ON HÆMOCHROMOGEN AND SOME RELATED COMPOUNDS

by

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It has long been known that if haemoglobin be treated with sodium hydroxide and a reducing agent haemochromogen is formed. This substance is of a bright rose colour, and has a two-banded absorption spectrum, of which the α band, 5578 A°.U., is so intense that its formation serves for the detection of small amounts of blood pigment. Bertin-Sans and de Moitessier (3) showed that pure haemin dissolved in sodium hydroxide does not give this reaction, though it does so in the presence of many proteins or other suitable nitrogenous compounds. Their work was confirmed by Anson and Mirsky (1), while in the formation of pyridine haemochromogen it was shown independently by Von Zeynek (11) and by Hill (4) that two molecules of pyridine combine with one molecule of reduced haematin. Haemochromogens are dissociated on dilution of the solution or on decrease in the hydroxyl ion concentration, and those having nitrogenous constituents of small molecular weight are precipitated from their solution after a short time.

This paper describes some experiments carried out in order to ascertain whether the reactions with reduced haematin would disclose any differences in the structure of certain proteins. It consists of four parts. (1) A study of the relative dissociations of the haemochromogens of several simple nitrogenous substances. (2) A determination of the quantitative relationships between reduced haematin and certain proteins. (3) Some experiments on the power of native and of certain modified proteins to form haemochromogens. (4) Some observations on parahaematins (compounds of haematin with proteins and other nitrogenous substances).

(1) The titration of several simple bases with reduced haematin was carried out spectrophotometrically. Since it was desired to use as concentrated solutions as was conveniently possible, a cell was constructed in which the thickness of the liquid was only 1 mm. It was filled, emptied, and washed by means of a capillary pipette. To delay the aggregation and precipitation of the haemochromogen molecules a protective colloid was added. For this purpose gelatine

was found most suitable, and was used in a concentration of 0·5%. The amount of haemochromogen due to its presence was negligible, and though it decreased the dissociation of the various haemochromogens to a small extent, this did not, however, affect the relative positions of the curves shown or the main conclusions to be drawn from them. The substances chosen were pyridine, α picoline, piperidine, methylamine, 4 methyl iminazole, and potassium cyanide. The procedure in each case was as follows: 13·0 mgm. of crystalline haemin was dissolved in 20 cc. of 0·5N sodium hydroxide; 0·4 gm. gelatine was dissolved in 20 cc. of the same soda. A suitable amount, found by trials, of the base was dissolved in 25 cc. of the same 0·5N alkali. In the case of methylamine the hydrochloride, and in the case of 4 methyl iminazole the oxalate, was used instead of the free bases. In each of a series of twelve tubes were placed 1 cc. of haemin solution, 1 cc. of gelatine solution, varying known amounts of the base, and sufficient 0·5N sodium hydroxide to make a total volume of 4·0 cc. To each tube in turn was added a few mgm. of sodium hydrosulphite, and a sample of the liquid was placed in the cell. After waiting a few minutes, in order to ensure complete reduction of the pigment, the intensity of the α band of the haemochromogen was determined on the photometer. The wave-length used was 5578 A°.U., except in the case of potassium cyanide, where it was 5680 A°.U. Owing to the fact that the maximum intensity of the α band of the cyanhaemochromogen is less than that of the others, this titration is decidedly less accurate. Accordingly another determination of the ratio between potassium cyanide and reduced haematin was made, using stronger solutions and reading on 5890 A°.U. At this wave-length the specific absorption of cyanhaemochromogen is much lower than that of reduced haematin. From this determination it was calculated that 1·93 molecules of potassium cyanide combine with one of reduced haematin. Within the limits of experimental error this agrees with the value found by Hill for pyridine. The S-shaped nature of the curves was pointed out by Hill, who considered it to be caused by polymerization of one or both of the pigments involved in the equilibrium.

From the curves the effect of various nitrogen linkages on haemochromogen formation can be seen. Most of the haemochromogens are far more dissociable than, for example, that of denatured globin. The relatively small dissociation of cyanhaemochromogen is noticeable, as is the high one of methyl iminazole haemochromogen. If the iminazole ring be responsible to any great extent for

LEGEND FOR FIGURES 1 & 2.

Ordinates: % haemochromogen formation.

Abscissae: No. of equivalents of base to one equivalent of reduced haematin, one equivalent=two molecules.

Curve 1: A, potassium cyanide; B, pyridine.

Curve 2: A, pyradine; B, 4 methyl iminazole; C, piperidine; D, α picoline; E, methylamine.

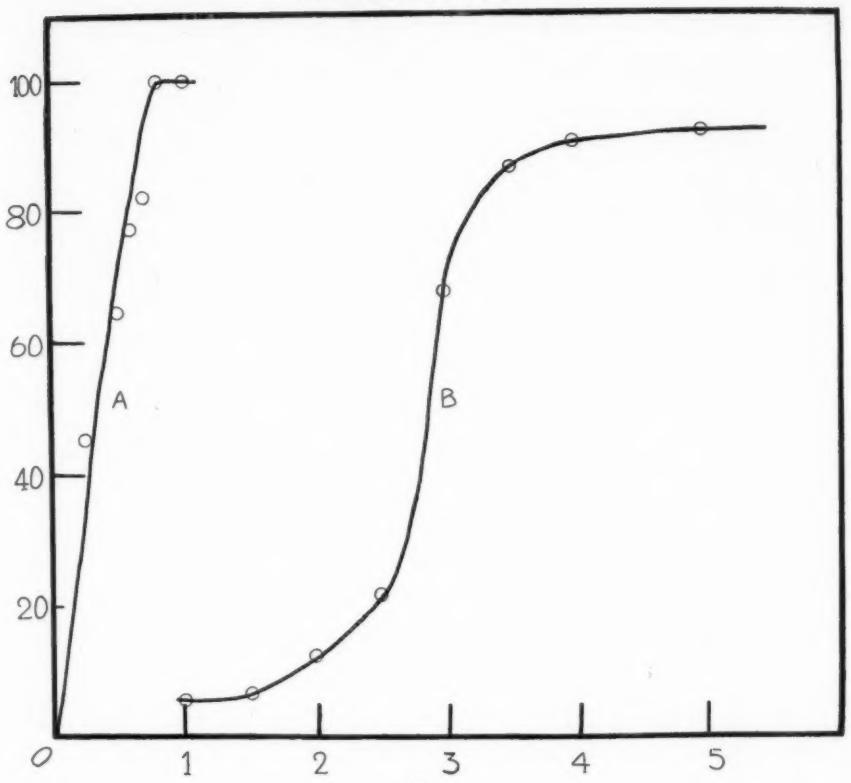
100
80
60
40
20
0
1
2
3
4
5
A
B

Fig. 1.

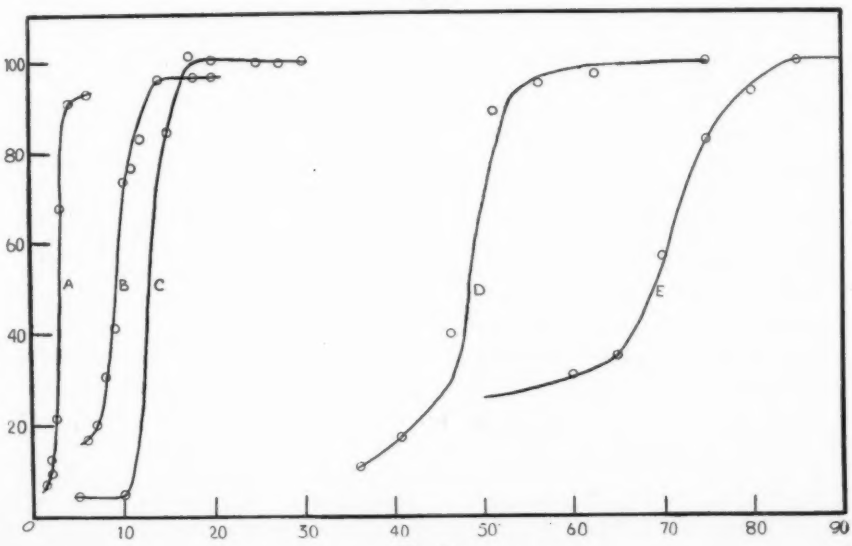
100
80
60
40
20
0
10
20
30
40
50
60
70
80
90
A
B
C
D
E

Fig. 2.

the haemochromogen of denatured globin the degree of dissociation must be profoundly modified by spatial arrangements in the protein molecule.

(2) In a previous paper (5) it was pointed out that denatured globin can combine with several times the weight of reduced haematin with which an equal weight of globin can combine. Using the spectrophotometer and the technique described in that paper, the amounts of various proteins that combine with unit amount of reduced haematin have been determined. The effect of haemochromogen dissociation was thus brought as low as possible. The proteins employed are given in Table 1. The egg albumin was twice crystallized by Hopkins' (6) method, and was denatured by treating in 1% solution with 0·2N. or 0·5N. hydrochloric acid at 40°C. for 17 hours. Both samples gave the same titration value with reduced haematin. It was precipitated by bringing to its isoelectric point, washed with water, and dissolved in dilute sodium hydroxide. A portion of the solution was used for protein estimation as described in the paper by Hopkins *(loc. cit.)*, and the remainder was made up to a suitable concentration of 0·5N. sodium hydroxide immediately before use. This protein was not dried, as upon drying it was found to undergo changes in its solubility, and hence further chemical changes were suspected. The casein was prepared from separated milk, dissolved, and reprecipitated. It was finally thoroughly extracted with ether, and kept as a dry powder till required. The dephosphorized casein was prepared according to the directions given by Rimington and Kay (9), using sodium hydroxide. The serum proteins were prepared by fractionation with ammonium sulphate in half-saturated solution. The globulin fraction was separated into euglobulin and pseudoglobulin by prolonged dialysis, and the euglobulin removed by filtration. The globulins were denatured separately by heating in 1% solution in 0·01N. hydrochloric acid on a boiling water bath for 20 minutes. After bringing to the isoelectric point the proteins were filtered off and washed with water, alcohol, and ether. The solid denatured proteins so obtained remained readily soluble in very dilute ammonia, sodium carbonate, or acetic acid, thus showing a marked difference from egg albumin which has been treated at laboratory temperatures with organic solvents. The serum albumin was denatured by treating with 0·5N. hydrochloric acid at 40°C. for three days. It was observed that this protein was far more resistant to acid denaturation than any of the others. After drying with alcohol and ether the denatured albumin remained soluble in the same reagents as the denatured globulins.

TABLE 1.

Protein.	Weight combined with one part by weight of reduced haematin.
Denatured ox globin	3.9
Denatured egg albumin	9.6
Denatured sheep serum albumin	7
Denatured sheep serum pseudoglobulin	19.5
Denatured sheep serum euglobulin	29
Casein	9
Dephosphorized casein	12

Owing to dissociation the globulins were read like potassium cyanide on 5890 A°.U., using more concentrated solutions.

(3) An examination was made of some native proteins to determine if possible whether denaturation was essential to the formation of a haemochromogen. As far as possible the conditions of testing for haemochromogen formation were arranged with a view to minimizing adventitious denaturation. O·1N. sodium carbonate was used as a solvent and sodium ferro-tartrate as a reducing agent. Even at this low concentration of hydroxyl ions the denatured proteins gave immediate and intense haemochromogen spectra, while their native originals in equal concentration gave none at first and only a feeble band on standing for some time. If much stronger solutions of the serum proteins, containing 2–3% of protein, were used, a faint band was immediately obtained. It was, however, no more intense than might be expected from denaturation of a trace of the protein during preparation. Fresh serum gives a very faint haemochromogen, but it undoubtedly contains a small amount of stroma protein in solution, which behaves as a denatured protein in this respect. Concentrated solutions of native proteins can retain in solution considerable amounts of denatured proteins even at the isoelectric point of the latter. A dilute solution of gelatine can dissolve nearly as much denatured sheep serum pseudoglobulin as there is gelatine present. Considerable caution must therefore be exercised in interpreting results obtained where mixtures of proteins are possible. In the case of egg albumin definite evidence was obtained that the concentration of sodium carbonate used does after some time produce enough denatured egg albumin to yield a precipitate when the solution is brought to its isoelectric point. In this connection it may be noted that freshly prepared casein and stroma proteins behave like denatured proteins.

Several proteins and other substances were treated with formaldehyde or with nitrous acid. Their ability to form haemochromogens after one or other of these treatments was examined. Among the simpler bases formaldehyde dissociated the haemochromogens depending on a primary or secondary amino group, such as those of methylamine and piperidine. On pyridine haemochromogen it had no effect. In the presence of formaldehyde pyridine immediately combined with reduced haematin. Hexamethylene tetramine haemochromogen is much less dissociated than that of ammonia. Hence when formaldehyde is added to a solution containing ammonia, reduced haematin, and ammonia haemochromogen, an increase in the intensity of the haemochromogen spectrum is observed, and a corresponding decrease in the intensity of that of reduced haematin. 4 methyl iminazole and β iminazolyl ethylamine (histamine) are of interest from their relation to histidine, which forms some 10% of the globin molecule. On neither of these bases had formaldehyde any effect as regards its ability to combine with reduced haematin.

Formaldehyde dissociated the haemochromogens of the proteins investigated very readily, with the exception of that of denatured globin, on which the effect

was only partial. Samples of the proteins were dissolved in dilute alkali and treated with a large excess of formaldehyde. The solutions were neutralized, and the precipitated protein well washed with cold water. In redissolving in dilute sodium hydroxide haemochromogens were readily obtained, showing that the formaldehyde-protein compounds were dissociable. In all the experiments with formaldehyde a considerable excess of the base or protein was always used, and sodium ferro-tartrate served as a reducing agent.

In the experiments on the effect of nitrous acid, where possible the substance was dissolved in dilute acetic acid, and a strong solution of potassium nitrite was added drop by drop. When this was impossible, as in the case of casein, the protein was dissolved in dilute sodium hydroxide solution and the potassium nitrate added. The acetic acid was then added in 10% solution drop by drop. The experiments with casein will serve as an instance of the quantities used. Five c.c. of 2% casein in 1% sodium hydroxide were treated with 1 c.c. of 25% potassium nitrite solution. Two c.c. of 10% acetic acid were added in single drops, and the mixture allowed to stand at room temperature for 20 minutes. The precipitate of deaminized casein was well washed with distilled water. In spite of extensive washing the precipitate of a deaminized protein is apt to retain mechanically small amounts of nitric oxide. The very great affinity of this substance for reduced haematin was at first the case of some confusion, as it inhibited all haemochromogen formation under the conditions of the experiments. It was, however, entirely removed by gently aerating either the precipitated protein suspended in water or its solution in 0·1N. sodium hydroxide. On testing the solutions of deaminized proteins with haemin dissolved in alkali and sodium hydrosulphite, it was found that a considerable proportion of the groups responsible for haemochromogen formation had been destroyed. In the case of casein about 25%, and in that of denatured ox globin 40%, remained. Among the simple bases the primary (from which the nitrogen is removed) and the secondary (which are converted into nitrosamines) gave no haemochromogens after this treatment; the tertiary were unattacked. 4 methyl iminazole is apparently quite unaffected by nitrous acid. In view of this it is difficult to believe that the iminazole ring of histidine is the sole or even the chief group in proteins which combines with reduced haematin. It may well be, especially in the case of denatured globin, that some of the molecules of reduced haematin combine with two different types of nitrogen atom, one of which is in the iminazole ring.

(4) When oxyhaemoglobin is denatured at the neutral point a red substance is formed, insoluble in water in the presence of salts, and having an absorption spectrum with two rather ill-defined bands nearer the violet end of the spectrum than the corresponding bands of oxyhaemoglobin. This is considered to be a compound of haematin and denatured globin. It has been described under the names of "Kathaemoglobin," "neutral haematin," and "parahaematin." Under the last name a discussion of the substance and its constitution occurs in

a paper by Keilin (7). Through him it has become clear that it is one of a class of substances, and that haematin, like reduced haematin, can combine with a large number of nitrogenous substances. The red substance with a two-banded spectrum, obtained by dissolving haematin in pyridine, is of this class. If the pyridine be carefully freed from impurities the parahaematin spectrum persists after removing all the oxygen from the solution by evacuation. Hence it is not, as Anson and Mirsky *(loc. cit.)* believed, an oxy-compound containing reversibly dissociable molecular oxygen. Like that of denatured globin, pyridine parahaematin is dissociated by the addition of small quantities of alkali. It is only formed in high concentrations of pyridine, 10% or over, and hence is seen to be a far more dissociated compound than pyridine haemochromogen.

The mixed proteins of sheep serum were denatured in 0·5N. hydrochloric acid for three days at room temperature. A solution containing about 3% protein was made in dilute sodium hydroxide and a small amount of haemin dissolved in it. The spectrum was that of alkaline haematin. On careful addition of 0·5% acetic acid a clear red solution was obtained at P.H. 7·8, whose spectrum showed the two bands of a parahaematin. It was not found possible to prepare parahaematins from denatured sheep serum, euglobulin, or pseudoglobulin. The haemochromogen value of these proteins is very low, as is seen from Table 1, and their haemochromogens are more dissociated than those of denatured globin or serum albumin. Thus the failure may be due, not to the absence of suitable groupings, but to the impossibility of preparing strong enough solutions to repress the dissociation of the parahaematin sufficiently for its spectrum to be observed in the presence of that of the haematin. On the other hand deaminized casein with a lower value than either of these proteins gives a parahaematin. As will be seen below, however, casein differs in some respects from the blood proteins. Some sheep serum albumin was denatured at 70°C. in 0·005N. hydrochloric acid for six hours. It was precipitated at its isoelectric point, and washed as free as possible from any residual native protein. After dissolving it in the minimum amount of alkali, and adding a small amount of haemin similarly dissolved, a fine red solution of denatured sheep serum albumin parahaematin appeared on cautious neutralization. Of the protein parahaematins which form in neutral solution, that of denatured sheep serum albumin is the easiest to prepare, since the isoelectric point of this protein is well on the acid side of neutrality. Native sheep serum albumin did not give a parahaematin.

Casein and denatured egg albumin differ sharply from the blood proteins in their behaviour with haematin. They form parahaematins only in strongly alkaline solution, and these parahaematins dissociate at the neutral point. Casein dissolved in 2% sodium carbonate or 1% sodium hydroxide gave no parahaematin, but on increasing the concentration of sodium hydroxide up to 3% a parahaematin was obtained with well-defined bands. A solution of 3% crystalline egg albumin in 5% sodium hydroxide, which denatures it rapidly,

gives a well-defined parahaematin, which, like that of casein, dissociates as the hydroxyl ion concentration is decreased. Deaminized casein and deaminized denatured egg albumin formed parahaematins, but deaminized sheep serum albumin did not.

Among the simple bases studied 4 methyl iminazole is the only one which forms a parahaematin in fairly dilute solution. Pyridine needs a concentration of 10% for fairly complete formation, while 0·25% suffices for 4 methyl iminazole. The contrast with their relative behaviour towards reduced haematin is very marked. The two iminazole bases studied show curious differences in this respect. Although under the conditions of titration their affinity for reduced haematin is about the same, the affinity of histamine for haematin is so low that its parahaematin was only to be obtained by allowing a solution of it and haematin to evaporate practically to dryness. On the other hand, a concentration of haematin of 0·59 mgm. and of 4 methyl iminazole of 3·4 mgm. per cc., i.e., 30 molecules of the base to 1 of haematin, give practically complete parahaematin formation.

The following table has an interest in showing how some earlier workers were led into a belief that casein or egg albumin could replace globin in oxyhaemoglobin.

TABLE 2.

Substance.	Parahaematin bands. A°.U. α	β
4 methyl iminazole	5,836	5,324
Denatured egg albumin	5,820	5,456
Casein	5,819	5,458
Ox oxyhaemoglobin	5,768	5,387

TABLE 3.

	Destruction of haemochromogen by KNO_2 and CH_3COOH.	Destruction of haemochromogen by CH_2O.	Formation of haemochromogen after removal of CH_2O.	Reaction at which parahaematin forms.	Destruction of parahaematin by KNO_2 and CH_3COOH.
Casein	Partial	Complete	Yes	Alkaline (3·4% NaOH)	Partial
Denatured egg albumin ..	Partial	Complete	Yes	Alkaline (2·5% NaOH)	Partial
Denatured sheep serum albumin	Partial	Complete	Yes	Neutral	Complete
Denatured sheep serum euglobulin	Partial	Complete	Yes		
Denatured sheep serum pseudoglobulin	Partial	Complete	Yes		
Denatured ox globin	Partial	Partial	Yes	Neutral	None
Methylamine	Complete	Complete	Yes		
Methyl iminazole	None	None		Neutral or alkaline	None
Piperidine	Complete	Complete	Yes	Neutral	Complete
Pyridine	None	None		Neutral	None

DISCUSSION.

Bertin-Sans and de Moitessier (3) first suggested how wide a range of substances can combine with reduced haematin to give typical haemochromogens. A further examination of nitrogenous compounds from this viewpoint has disclosed additional facts. Thus piperidine and methylamine do not combine with reduced haematin to any appreciable extent in sodium carbonate solution, even when the bases are present in high concentration. The effect of a neighbouring carboxyl group in inhibiting haemochromogen formation is well shown by a comparison of methylamine with glycine. A synthetic sample of the latter gave only the faintest band in moderately strong solution in 5% sodium hydroxide. Where a benzene ring is near the nitrogen of the base, as in aniline or quinoline, the haemochromogen is not formed. Diazo compounds, or diazo-amino compounds, do not form haemochromogens. Phenyl hydrazine haemochromogen is very much more dissociated than that of hydrazine. Histamine has the unusual property of showing a small degree of haemochromogen formation in a very low concentration, 0·0025%. 4 methyl iminazole could not be detected in a concentration ten times greater. For complete haemochromogen formation, however, these bases required about the same amounts.

The study of the reactions of haematin and of reduced haematin with the various proteins described in this paper suggests that more than one kind of group which can unite with these pigments occurs in proteins. From the work of Barcroft and his collaborators (2) it follows that there are probably many globins, since the oxyhaemoglobins of different species are chemically distinguishable. This class of proteins stands alone in its relations with these two pigments. No other type of protein has yet been found capable, on careful examination, of replacing globin in the respiratory blood pigment. Of the other native proteins examined none were able to combine with either haematin or reduced haematin. When the denatured proteins are surveyed their reactions with these pigments show certain interesting differences. Like denatured globin, but unlike their native originals, they form haemochromogens whose composition and degree of dissociation differ for different denatured proteins. Casein behaves, even when freshly prepared, as a typical denatured protein. An examination of a few parahaematins shows that while denatured blood proteins form them in nearly neutral solution, casein and denatured crystalline egg albumin only do so in the presence of a high concentration of hydroxyl ions. Whether or no the same group in a protein is responsible for parahaematin formation as for that of haemochromogen, it is hard to imagine that the group responsible for its formation at the neutral point and dissociation in alkaline solution is the same as that in a compound whose parahaematin is formed and dissociated under the opposite conditions. No simple substance is known whose parahaematin is formed exclusively in strongly alkaline solution. 4 methyl iminazole parahaematin is not affected by moderate amounts of alkali.

Any satisfactory theory of protein denaturation must account for these differences between native and denatured proteins. On the basis of the accepted value, for egg albumin, of a molecular weight of about 34,000, denaturation involves, *inter alia*, the exposure of 10 nitrogen atoms per molecule, of which only a few are tertiary. Mastin and Schryver (8), from a study of the action of nitrous acid on this protein, conclude that denatured egg albumin has the same number of primary amino-groups as native egg albumin. Their results may in part be attributed to the fact that nitrous acid to some extent denatures this protein. Whether all the nitrogen atoms set free on denaturation of egg albumin which combine with reduced haematin, and which are attacked by nitrous acid, are secondary must remain at present an open question. An analysis of deaminized casein by Skraup and Hoernes (10) showed a great decrease in lysine, which is perhaps not without significance in this connection. Denaturation is seen to consist of a number of reactions occurring in different portions of a protein molecule. It is probable that most denatured proteins consist of mixtures, and that different methods of denaturation produce end-products differing in certain physical characteristics. In some proteins at least sulphur groupings are also involved in the change. These considerations should emphasize the need for caution in accepting without further evidence the view that denaturation is a process readily reversible by simple physico-chemical methods.

The authors desire to record their indebtedness to Professor W. A. Osborne, Professor of Physiology in the University of Melbourne, for the loan of the spectro-photometer used in these experiments.

SUMMARY.

1. Determinations have been made of the relative dissociations of haemochromogens containing nitrogen in different states of combination.

2. The quantitative relationships between reduced haematin and certain denatured proteins have been estimated.

3. Some haemochromogens are dissociated by formaldehyde.

4. Native proteins do not form haemochromogens.

5. Some parahaematins are described.

6. The effect of nitrous acid on the ability of proteins and some other nitrogenous substances to form haemochromogens and parahaematins is described.

REFERENCES.

1. Anson, M. L. and Mirsky, A. E.: Journ. Physiol., 60 (1925), p. 50.
2. Barcroft, J., Anson, M. L., Mirsky, A. E. and Oinuma, S.: Proc. Roy. Soc., 97B (1925), p. 61.
3. Bertin-Sans et de Moitessier: C.R. Acad. Sci., 116 (1893), p. 401.

4. Hill, R.: Proc. Roy. Soc., 100B (1926), p. 419.
5. Holden, H. F. and Freeman, M.: Aust. Journ. Exper. Biol. and Med. Sci., 5 (1928), p. 213.
6. Hopkins, F. G.: Journ. Physiol., 25 (1900), p. 306.
7. Keilin, D.: Proc. Roy. Soc., 100B (1926), p. 129.
8. Mastin, H. and Schryver, S. B.: Biochem. Journ., 20 (1926), p. 1177.
9. Rimington, C. and Kay, H. D.: Biochem. Journ., 20 (1926), p. 777.
10. Skraup and Hoernes: Monatshefte f. Chemie, 27 (1906), p. 631.
11. Von Teynek, R.: Zeits. Physiol. Chem., 70 (1910), p. 24.

A TITRATION METHOD FOR THE DETERMINATION OF POTASSIUM IN THE URINE

by

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In attempting numerous potassium estimations on urine we have been confronted with the fact that most of the suitable methods require preparatory ashing. Fisk, in a preliminary communication, proposed a double precipitation to avoid this time-consuming operation. After some experimenting we found that it was possible to apply the perchlorate method of Schloesing-Wense to the unashed urine.

PRINCIPLE.

The potassium is precipitated from the unchanged or concentrated urine with an excess of perchloric acid. The potassium perchlorate precipitate is washed with alcohol. Afterwards the potassium perchlorate is titrated with a standardized solution of methylene blue, using the dye added as self indicator.

PREPARATION OF REAGENTS.

(1) Perchloric acid. Use about 50% perchloric acid. If this is not available commercial 20% perchloric acid is concentrated to approximately one-fifth of its original volume. Towards the end of the evaporation heavy fumes of perchloric acid are given off, and crystals may appear on cooling. The resulting acid has a concentration of about 40–50%.

(2) Methylene blue solution. 32 grammes of commercial methylene blue (we found Merck's B extra satisfactory) are dissolved in 1 litre of distilled water by warming. Let stand overnight and filter. Keep in brown glass bottles, and add a few drops of chloroform. We found this solution stable for at least one month. The methylene blue solution is standardized against a perchloric acid solution of known strength by titration as described in the following paragraph (1 cc. of this solution will be equivalent to about 2·5 mgm. of potassium).

(3) Alcohol. Commercial absolute alcohol.

(4) Disodium phosphate, ground.

PROCEDURE.

Owing to the wide variation in the potassium content of different urines, and depending to some extent on the amount of urine available, different procedures have to be adopted to obtain the best results.

If there is only a small amount of urine of apparently low gravity available (20 cc. or less) it is best to transfer from 5–20 cc. of urine into a Folin tube or beaker, and to concentrate to about one-fifth of the original volume, avoiding charring. Then transfer the concentrated urine to a 15 cc. centrifuge tube by washing the Folin tube or beaker several times with small amounts of absolute alcohol, making up the volume with absolute alcohol to about 12 cc., and add 1 cc. of concentrated perchloric acid.

If a larger amount of urine is available, put 5 cc. of urine in a centrifuge tube and add 1 cc. of perchloric acid. If a considerable precipitate of potassium perchlorate appears immediately no further concentration will be necessary; this is all that will be required for many normal urines. On the other hand, if only a scanty or no precipitate appears the urine has to be concentrated either as a whole or on an aliquot part, as described above. Volume is made up to about 13 cc. with absolute alcohol. Mix and let stand overnight, preferably in a refrigerator.

In urines containing a large amount of protein the proteins should be removed before the addition of the perchloric acid.

Occasionally on adding perchloric acid to urine, particularly if the urine is fresh, a colour ranging in different cases from pink over purple to blue appears, and sometimes also a very small amount of a coloured precipitate is noticed.

After standing, centrifuge and pour off the supernatant fluid; drain as described by Clark and Collip in their method for calcium determination. Wash with 2 cc. of alcohol, taking care that the precipitate is rinsed thoroughly with the wash fluid. Centrifuge and drain again. A second washing with about 1 cc. af alcohol is advisable on account of the frequently persistent presence of considerable traces of perchloric acid.

The white precipitate is transferred with small amounts of water to a strong beaker of about 100 cc. capacity. The volume of the solution should not exceed 10 cc., and in the case of a small precipitate should not exceed 5 cc. Then add about 2 grammes of ground disodium phosphate and mix thoroughly.

From a 25 cc. burette add the standardized methylene blue solution under constant stirring with a glass rod. The first few cc. added (in the case of an average amount of potassium) will combine immediately with the potassium perchlorate present, forming a crystalline purplish precipitate of methylene blue perchlorate. Owing to the excess of potassium perchlorate and disodium phosphate present the methylene blue perchlorate does not go into solution until the end point is nearly reached. At this stage the purple precipitate takes a somewhat bluish colour. The end point is reached after all the potassium

perchlorate present has been combined with the methylene blue, and any excess of methylene blue makes itself evident by giving the solution the typical colour of methylene blue. But by this method the appearance of the end point could hardly be accurately determined and a spotting method on filter paper has been used. We used filter paper Whatman No. 40. At the distinctly purple precipitate stage it is hardly necessary to make the spotting test. But if one puts a drop of the mixture on the filter paper with the glass rod used for stirring the methylene blue, perchlorate shows itself as a dark spot, consisting of methylene blue perchlorate crystals, while the entirely colourless solution forms a watery ring around it, due to the spreading of the solution on filter paper. A few tenths of a cc. before the end point is reached a small, pale greenish coloured ring is formed around the precipitate of methylene blue perchlorate on the filter paper. The end point is reached as soon as the coloured ring has a distinct bluish appearance.

For the proper recognition of the end point thorough stirring of the mixture is essential. If one is doubt whether the end point has been obtained it is recommended to bring a large drop on to the filter paper (about 0·8 cm. diameter of the crystalline precipitate), which will produce a correspondingly large and distinct colour ring. The number of cc. of methylene blue used indicate the amount of potassium perchlorate present according to the previous standardization of methylene blue against a perchloric acid of known titre.

The standardization of the methylene blue against perchloric acid is done in the same manner as the titration of potassium perchlorate described above.

COMMENT.

The perchlorate method for the determination of potassium has been recommended for ashed biological material by W. C. Davis. Since then the macro-method has been used from time to time on physiological problems. Dienes applied the principle to micro determinations by using micro-apparatus. The direct potassium perchlorate precipitation of potassium in inorganic salt solutions without previous evaporation has been used by Crotognio. Our object in applying the potassium perchlorate method to urine has been to avoid ashing of the urine and weighing of the potassium perchlorate precipitate.

Since the potassium perchlorate is soluble to some extent the application of a concentrated perchloric acid was advisable. Further, urines with a low potassium content have to be concentrated. The precipitate may be separated and washed either by centrifugalization or by filtering through some micro-filter arrangement. We chose the centrifugalization method because the precipitate is well suited for this procedure, and does not float, as is found to be the case occasionally with the sodium potassium cobalt nitrate precipitate in certain other potassium methods.

The property of perchlorates to form more or less soluble salts with organic compounds led to the introduction of analytical methods based on this principle.

Fichter and Jenny proposed a gravimetric method for the determination of perchlorates by precipitation with nitron.

Monnier described a colorimetric method for approximate estimations of perchlorates in Chili saltpetre by methylene blue. For the determination of potassium in urine we chose the perchlorate method combined with the methylene blue precipitation because the colouric properties of methylene blue allowed us to devise a relatively simple estimation by titration.

The underlying chemical process may be formulated as follows:

$$\text{Methylene blue} + \text{potassium perchlorate} \rightleftarrows \text{Methylene blue perchlorate} + \text{potassium chloride.}$$

In neutral medium with a considerable excess of potassium perchlorate still present all the methylene blue added combines immediately with the potassium perchlorate, forming a crystalline precipitate of purple appearance. Near to the end point there seems to be a tendency for the methylene blue perchlorate to go into solution and also to dissociate to a slight extent, which makes the observation of the end point difficult. In acid medium solutions dissociation of the methylene blue perchlorate occurs under any condition; a weakly alkaline medium seems to furnish the best conditions, and for this purpose disodium phosphate is added in a large excess.

The method as described above is rather a semi micro-method, giving the most satisfactory results with amounts from 3–80 milligrams of potassium.

Although the methylene blue titration hardly reaches the accuracy of the ordinary standard titration methods, we considered it sufficiently accurate to be used on a biological material such as urine, in which the variations of potassium content are large.

The errors inherent to the method, the slight solubility of potassium perchlorate on one side, and the excess of methylene blue necessary for recognition of the end point on the other, compensate one another to a certain extent.

As can be seen in Table 1, we were able to recover known amounts of potassium chloride from urine with an error of $\pm$ 2%. Further, we checked the method on a normal urine in which the potassium content had been determined by the macro method of Schloesing-Wesne (Table 2).

Known amounts of potassium in salt mixtures containing all the electrolytes in the proportions found in normal urines were determined with a fair degree of accuracy (Table 3).

This method, which has so far only been applied to urines, could also be utilized for other biological materials containing not too small an amount of potassium.

SUMMARY.

1. A simple semi-micro method for determination of potassium in urine has been described.

2. Potassium is precipitated from the unashed urine as potassium perchlorate; the potassium perchlorate is titrated with methylene blue.

TABLE 1.

Recovery of Potassium added to Urine.

Potassium present. grm.	Potassium chloride added. grm.	Total potassium present. grm.	Total potassium found. grm.	Error.
.381	1.0	.905	.905	— .2%
.381			.895	—1.1%
.381	2.0	1.429	1.435	+ .4%
.381			1.404	—1.0%
.381	3.0	1.953	1.985	+1.7%
.381			1.991	+2.0%
.381	4.0	2.477	2.452	— .6%
.381			2.418	—2.4%

TABLE 2.

Potassium Content of Urine determined by Titration Method described and by the Gravimetric Method (Schloesing-Wense).

Potassium found by the author's method. grm.	Potassium found by gravimetric method (Schloesing-Wense). grm.
6.800	6.79
6.750	6.88
6.842	

TABLE 3.

Potassium Determinations on Salt Mixtures as found in Urines.

Potassium present in 100 cc.	Potassium found in 100 cc.	Remarks.
1.572	1.60	Concentrated
	1.56	5x. determination
	1.61	on 3 c.c.
2.620	2.65	Not concentrated
	2.63	determination
	2.63	on 5 c.c.

REFERENCES.

1. Clark, E. P. and Collip, J. B.: Journ, Biol. Chem., 63 (1925), p. 461.
2. Crotognio, F.: Zertschriftfuer analyt. Chemie, 57 (1918), p. 478.
3. Davis, W. A.: Journ. Agric. Sci., 5 (1912), p. 53.
4. Dienes, L.: Journ. Biol. Chem., 61 (1924), p. 73.
5. Fichter, Fr. and Jenny, E.: Helv. chemica acta., 6 (1923), p. 227.
6. Fisk, C. H. and Litarczek, G.: Journ. Biol. Chem., 67 (1926), p. 16.
7. Monnier, A.: Chem. Zentrbl., 88 (1917), p. 132.
8. Wense, W.: Zeitschr. f. angew. Chemie., 6 (1892), p. 233.

SYSTEMIC VENOUS PRESSURE

by

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(Submitted for publication 15th May, 1929.)

The pressure in the systemic veins must naturally be still lower than that in the capillaries if circulation is in progress and in the normal direction. Owing to the high extensibility of the vein wall relatively large increases in capacity correspond with but slight increases of pressure. The most obvious changes in venous pressure are those conditioned by respiration; as might be expected, these are most marked in the thorax. The aim of the present research was to determine what effects are produced on systemic venous pressure by altered conditions of heart and arteriole. It was found that, granted undisturbed cardiac action, the venous pressure remains remarkably constant. This constancy may be ascribed in part to the resistance and capacity of the capillary bed, to the special qualities of the vein wall, and to the Bainbridge reflex, whereby quickening of the heart is produced by rise of pressure in the adjacent great veins.

METHODS.

Morphinized dogs were anaesthetized with chloroform, and were curarized, chloroform air being administered by a pump in the usual manner. The carotid pressure was recorded by a U-tube mercury manometer of the ordinary pattern with float. The femoral vein in Scarpa's triangle on one side was ligatured distally and cannulized with the cannula pointing heartward. It is obvious that this must be subject to the pressure in the corresponding iliac vein. In some cases the cannula was long enough to be thrust up into the iliac close to the cava; in other cases the cannula did not extend above Poupart's ligament, but no difference could be detected. The cannula and the tubing connected therewith were filled with magnesium sulphate solution of density 1·117. Various devices were tried to get records by allowing a float to ride on the surface of the magnesium sulphate solution, but all proved unsatisfactory. Eventually excellent results were obtained by connecting the vein cannula by means of magnesium sulphate solution with a vertical tube of 4·5 millimetres internal diameter, in which the level of the solution was well below the top of the tube, and was obviously almost on a horizontal line with the cannula. The air space above the magnesium sulphate

meniscus was connected by a simple piece of rubber tubing with a Marey air tambour vertically above. The length of the tambour recording lever could easily be adjusted so that the excursions of the writing point, at least in the middle register, equalled the vertical excursions of the magnesium sulphate meniscus. In the tracings illustrating this article the lever movements were nine-tenths of the fluctuations of the magnesium sulphate solution. If the density of mercury is taken as 13·6, and if allowance is made for the fact that the excursions of the mercury manometer are half the actual, the venous tracing therefore has a sensitiveness 22 times that of the carotid arterial record. The venous lever was placed vertically over the arterial. In the tracings time is registered in seconds, and a scale with centimetres at the left margin gives the reduction in size. From time to time the system connected with the vein was tested to ensure that clotting or other obstruction had not occurred.

Results in the unc urarized dog were identical with those given here, except that asphyxia and nociceptive reflexes were not attempted.

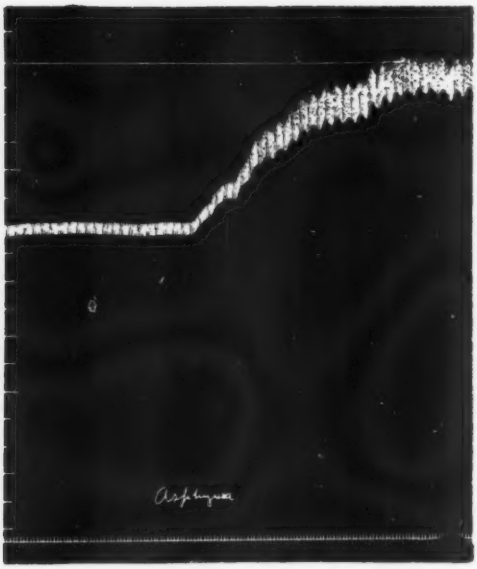

Fig. 1.

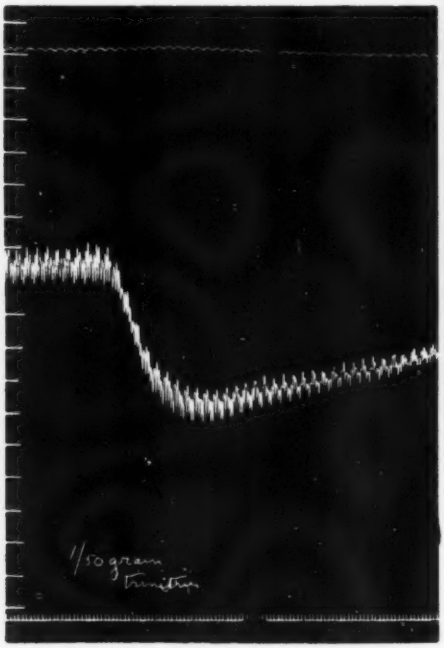

Fig. 2.

EXPERIMENTS.

Figure 1.

Here there is a marked rise of arterial pressure in a curarized vagotomized dog, due to asphyxia. It will be observed that though a major change has taken place in the systemic arterial circulation, no disturbance is observable in the venous record. Rise of arterial pressure also produced reflexly by nociceptive stimulation was also without effect on the venous pressure.

Figure 2.

A fall of arterial pressure was produced by intravenous injection of 1/50 grain trinitrin. A very slight rise in the venous pressure can be observed.

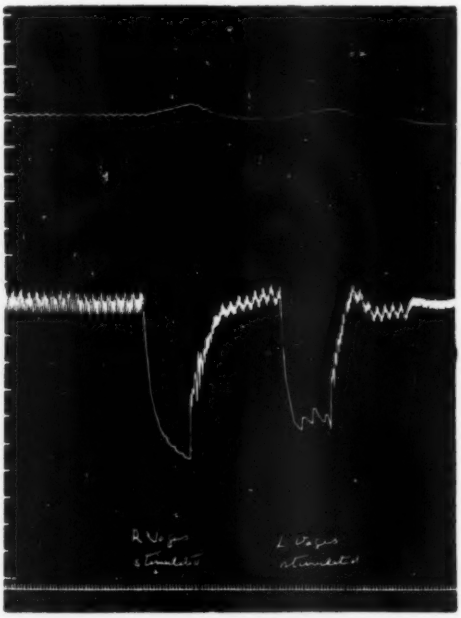

Fig. 3.

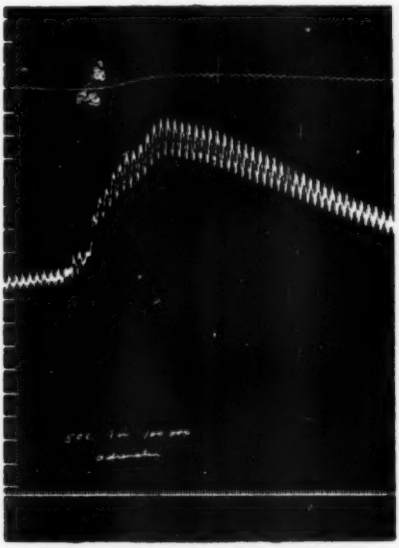

Fig. 4.

Figure 3.

Stimulation of the peripheral end of the cut vagus on each side gave syncopal fall of arterial pressure. The venous tracing shows a decided rise. It is remarkable how quickly this venous effect is developed, considering the distance of the femoral vein from the heart.

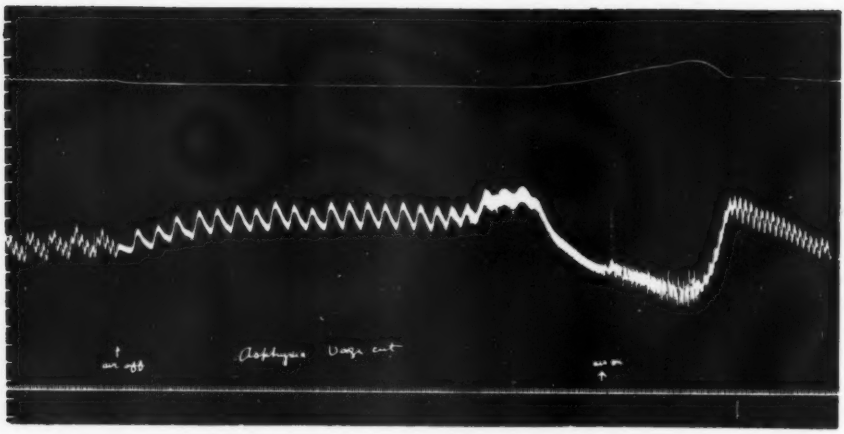

Fig. 5.

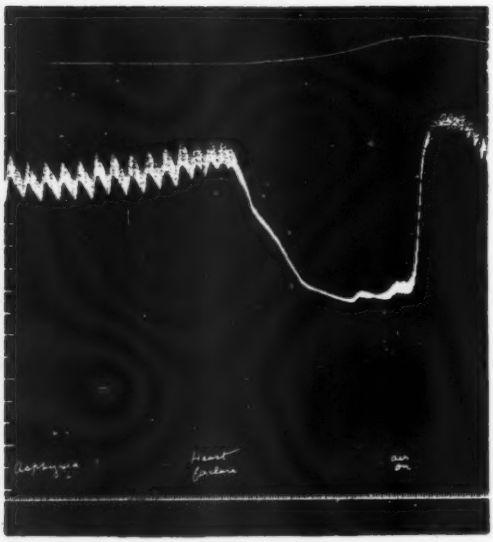

Fig. 6.

Figure 4.

This is chosen as fairly typical of the action of adrenalin in a vagotomized dog. In many cases the venous rise was scarcely observable. Generally speaking the more obvious the signs of cardiac embarrassment in the arterial record the greater was the rise of venous pressure. Indeed, my experiments lead me to

the belief that rise of venous pressure when it occurs with adrenalin is chiefly due to the heart being overtaxed. When adrenalin is administered to a dog with vagi intact the venous pressure shows a well-defined rise, but here vagal heart slowing is present.

Figures 5 and 6.

Asphyxia in a curarized, vagotomized dog was allowed to continue until heart failure was manifest. Artificial respiration was then restored, bringing about recovery of the heart, as shown by the quick rise of arterial pressure. It will be noted that throughout the period of change of arterial pressure due to the asphyxia no venous alteration can be seen until the heart succumbed in the rapid manner it usually displays in such circumstances. Restoration of strength by supplying oxygenated blood leads to a depletion of the engorged caval veins and a corresponding fall of venous pressure.

Figure 7.

It will be observed in this interesting tracing that asphyxia in the vagotomized dog led to the usual rise of arterial pressure, but the venous pressure remained a considerable time unaltered. Then rather suddenly the venous pressure rose, though the arterial pressure was still high. That the heart was embarrassed is shown by the quick rise of arterial pressure when the artificial respiration was resumed. The heart is the first organ to get the oxygenated

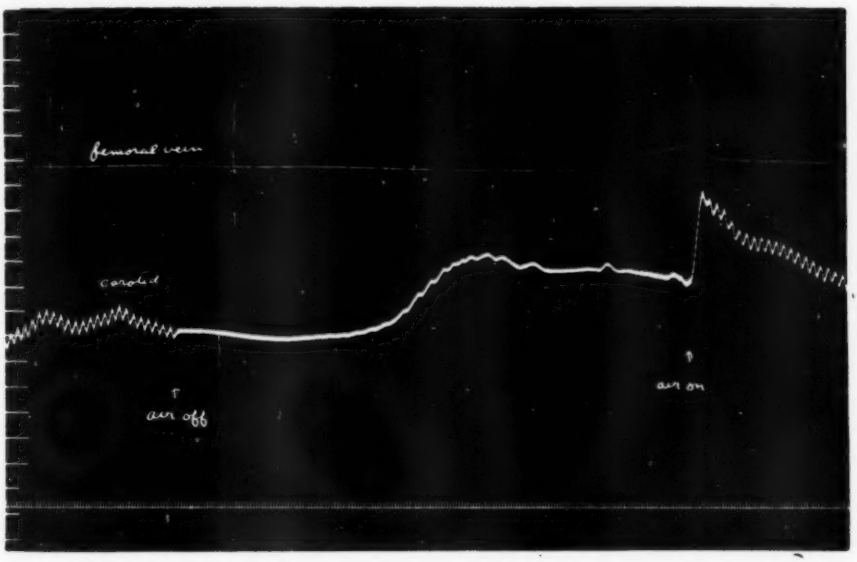

Fig. 7.

blood, and beats strongly against a high resistance; then as the oxygenated blood reaches the medulla oblongata the arteriolar constriction relaxes and the arterial pressure falls.

CONCLUSIONS.

The venous pressure, so long as the heart is normal, is wonderfully constant in the absence of muscular exertion. Major changes can occur in the systemic arterial pressure without affecting the venous. If the heart be embarrassed by anoxaemia, or by having to inject blood into the aorta against too great a resistance, or if it be slowed by vagus action, the venous pressure undergoes a definite augmentation fairly quickly established.

PHYSIOLOGICAL DERANGEMENTS IN VINES SUBSEQUENT TO INJURY BY COLD

by

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(From the Department of Botany, University of Adelaide).

(Submitted for publication 16th April, 1929.)

The object of this paper is to place on record the nature of the pathological symptoms caused in currant and sultana vines by the "Black Frost" in the irrigation areas in the Murray Valley in September, 1927. The injury was of a twofold nature: first, the destruction of the living tissues of the shoots immediately following the frost, and, secondly, the subsequent falling of the flowers produced from dormant buds. Observations of the damage were made and material collected at Renmark, Berri, Monash, and Barmera, in South Australia, and at Mildura and Red Cliffs in Victoria, which places the author visited in order to prepare a report for the Commonwealth Council for Scientific and Industrial Research.

Nature of the Frost.

The damage was not caused by a frost in the ordinary sense of the word, but by a shallow drift of cold air, which rolled over the country just before sunrise on September 25, 1927. This drift extended about 5 feet above ground level, and came from the south-west. The severity of the cold varied from place to place. At Renmark, generally, 8–9°F. of frost were recorded, at Berri 9°, and at Mildura 10–12° of frost. Investigations showed that the contour of the land, which determined the length of time the drift would lodge in any particular spot, had a greater effect in causing injury than any other factor.

The soil temperatures remained fairly constant for the week in which the frost occurred. At Berri the minimum temperatures at 18 inches below the surface of the soil varied from 52° to 57°F. Consequently little damage was done to the root system of the vines; the tap and main lateral roots were unaffected, but a few shallow fibrous roots were killed.

Effect on the Vines.

The vines chiefly affected were sultanas and currants, since practically all the buds had produced shoots at the time the frost occurred. In all cases where

the plants were exposed to the cold for any length of time, not only were all green shoots and leaves killed, but also a great deal of the woody tissue. In currant vines, wood from 5–6 years old was extensively injured or destroyed, and the damage in sultanas also extended to the old wood. The nature of the injury is entirely different from that caused by ordinary or white frosts, in which damage is confined to leaves and young shoots, and is caused primarily by the rapid thawing of the tissues at sunrises, and consequent laceration of the cells.

In the present case microscopic examination of the injured tissues of the young green shoots showed that there had been a breakdown and killing of the parenchyma cells of the cortex, of the medullary rays, and of the pith, followed by torsion and laceration of the wood elements. This is a direct result of the low temperature.

Damage to the woody portions of the vines was extensive and of a serious nature. Examinations of many shoots showed clearly that the first effect of the frost was to cause a degeneration of the cambium of the stem. The cells of the cambium suffered severe oxidation metabolism, and the protoplasmic contents became brown, due to the formation of oxidized phenolic compounds, chiefly catechol-containing derivatives. Finally the cell contents completely degenerate, and the cell walls become broken. The cells of the cortex and of the phloem do not appear to have been injured primarily, but after necrosis of the cambium they are cut off from the vascular water supply, gradually lose water, and undergo a similar oxidation to the cambium, although the breakdown of their organization is not so rapid.

This degeneration of the cambium is a characteristic symptom in badly injured vines, in that the whole of the tissues beyond the cambium, including the bark, peel readily from the wood which is exposed. This is not possible in uninjured vines. Following the breakdown of the protoplasm of the cells of the outer tissues, air is admitted to the vessels and tracheids of the wood, which are thus rendered functionless for the conduction of water. There is no trace in

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EXPLANATION OF PLATE.

Fig. 1. Microphotograph of tranverse section of cane of sultana vine. In this section the damage is not extensive and the cambium cells show only a slight brown colouration. In cases of severe injury all the tissues below the cambium become isolated from the wood. A certain amount of tannin is found normally in the cells of the phloem, and can be seen as black accretions in the cells. The dark colour in the pericycle is not due to tannin, but to suberin, and marks the beginnings of the phellogen.

(Section stained with eosin and photographed in blue-green light. $\lambda=570\mu\mu$. Magnification × 65.)

Fig. 2. Microphotograph of transverse section of main axis of inflorescence of sultana vine. This photograph shows three stages in the onset of the disease. At (a) the cambium alone shows the beginnings of phenolic oxidation. At (b) the abnormal metabolism has spread by way of the medullary rays to the first layers of the pith, and also to the phloem and cortex. The cells have not yet degenerated. At (c) the cells have completely degenerated and have isolated the vascular bundles. The pith has degenerated.

(Section unstained; photographed in white light. Magnification × 65.)

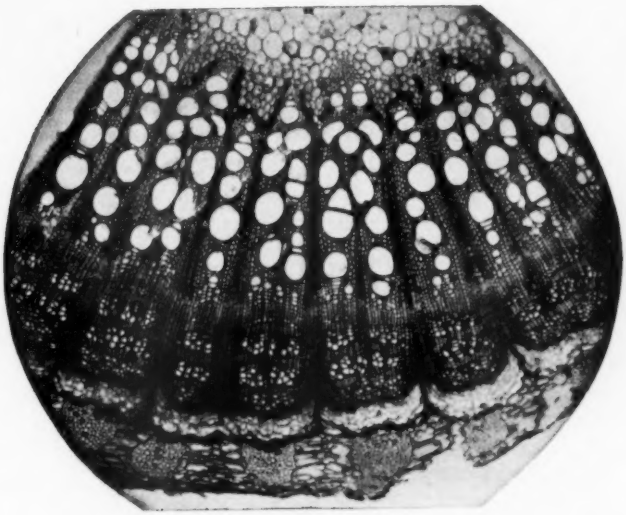

Fig. 1.

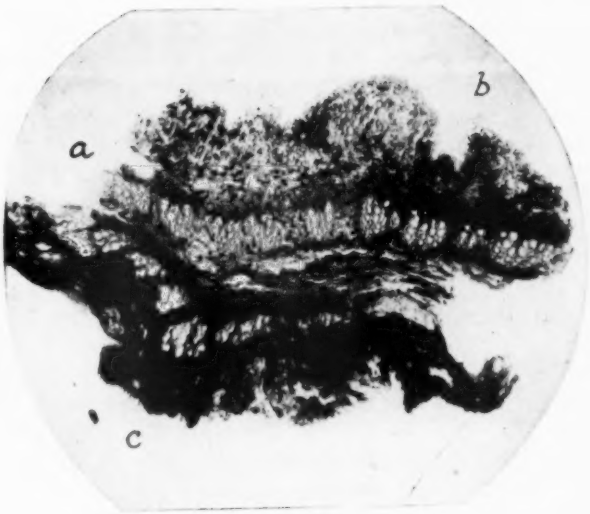

Fig. 2.

these shoots of the cells of the medullary rays becoming meristematic and forming callos tissue around the element of the xylem.

In cases where the whole shoot has not been badly injured by the frost, the cambium and cortical cells do not degenerate, but show traces of the oxidation of phenolic compounds, as is evidenced by the faint brown colouration produced in the protoplasm, due to substances which give reactions for polyhydroxy-benzenes. (See figure 1.)

It is the usual practice in pruning sultana vines to twist the cane in two or three places. This ruptures some of the vessels of the wood, and also the cells of the phloem and cortex. As a result of cambial activity callus tissue is formed at these points, and the normal passage to other parts of the plant of the carbohydrates manufactured by photosynthesis is restricted, so that they accumulate in the cane above the twists. This high concentration of carbohydrates in this portion of the cane is undoubtedly an important factor in the subsequent oxidative metabolism. It was noteworthy that the greatest amount of injury and degeneration of cells occurs in the part of the cane furthest from the main stem of the vine, that is in the region below which there are three or four twists. There are, of course, many cases in which the whole vine has been injured by the cold, but generally speaking the cells other than the cambium do not show such browning, and degeneration is seen in the canes themselves.

When the temperature rose after the temporary paralysis of cell activity by the cold it is evident that respiration did not follow the normal course, but was diverted, in particular, to the metabolism of hydroxy-aromatic compounds. This peculiar metabolism caused a further pathological condition about a month after the original injury by the frost. This was the falling of the flower from the branches bearing the flower clusters.

These inflorescences had been produced from dormant buds on wood or canes which had been only slightly injured by the cold, and in which the cambial cells show only a slight brown colouration. The flower buds opened in most cases, and then almost invariably dropped, leaving bare the main axis of the inflorescence. Sections of the axis at this stage showed that all the tissues appeared normal, except the cambium, which was beginning to brown, and on examination proved to contain tannin.

The second stage in the history of the disease is the appearance of a black spot or streak, which develops locally on the main axis. This streak spreads rapidly, until the whole inflorescence becomes dead and blackened. This streak is not caused by fungal attack, nor could any sign of insect puncture be detected, but is due to derangements in the normal metabolism of the cell.

The progress of the tissue destruction was followed by means of sections at different stages. (See figure 2.) The development of tannin in the cambium was followed by an intense metabolic activity, in which the pericycle formed large amounts of tannin. The cells of the medullary rays, and of the layer of

cells in the pith which are next to the xylem trachieds, followed and behaved in a similar fashion. All these cells later degenerate and die, so that the whole of the vascular tissue is isolated from the living tissue (see figure 1). Under these circumstances no sap can reach the flower buds, which die.

There has been a definite reaction in certain cells of the pith, particularly those next to the degenerated cells. These cells became meristematic, and divided rapidly, forming cells with cutinized walls. This protected the living cells of the pith from further degeneration, but at the same time cut them off from the vascular elements, so that they, too, gradually died.

The chief microscopic symptoms can be seen in figure 2, which is a micro-photograph of the main axis of the inflorescence. At the top of the section can be seen the earlier stages in the isolation of the xylem. In the lower portion of the section the disease is more advanced, and extensive degeneration of most living cells has occurred; the pith also has collapsed, and the xylem elements are completely isolated.

THE ACID REACTION AND CO_2 CONTENT OF CONDUCTIVITY WATER

by

RUPERT JETHRO BEST

(From the Chemistry Department, University of Adelaide).

(Submitted for publication 2nd May, 1929.)

Numerous investigators, from Walker and Cormack in 1900 to Kolthoff in 1926, have endeavoured to correlate the CO_2 content of "equilibrium water" with its conductivity. The results in most cases show a close agreement. Kolthoff (3) also calculated and measured its acid reaction. These investigators used as the basis of their calculations the percentage of CO_2 present in the air, and applied Henry's law. This method is not applicable to conductivity water containing less than the equilibrium amount of CO_2, that is, to water of specific conductivity (l) less than $0{\cdot}75 \times 10^{-6}$ mho. Kolthoff's values for equilibrium water will serve for comparison with the author's. Kolthoff calculated the pH of such water to be 5·7 and found the pH (colorimetrically) to be 5·7. Further, he calculated the CO_2 content to be $1{\cdot}5 \times 10^{-5}$ molar, and by titration obtained the value $1{\cdot}55 \times 10^{-5}$ molar.

During the course of investigations on colloidal solutions the author found it necessary to apply corrections for the pH of conductivity water of specific conductivity $\cdot 3 \times 10^{-6}$ to $\cdot 75 \times 10^{-6}$ mho and for its CO_2 content.

The CO_2 content of such water may be found by calculation from its specific conductivity by applying the following relationships:

$$\alpha = \frac{l.V}{\Lambda\infty} \quad . \quad . \quad . \quad . \quad (1)$$

$$k = \frac{\alpha^2}{(1-\alpha)V} \quad . \quad . \quad . \quad (2)$$

From these

$$V = \frac{k.\Lambda^2\infty}{k.l.\Lambda\infty + l^2} \quad . \quad . \quad . \quad (3)$$

k and $\Lambda\infty$ are constants and are known.

The value of l is determined by direct measurement, and hence the value of V corresponding to any value of l may be calculated for a monobasic acid; V can then be calculated to molality.

Practically the whole of the conductivity (over and above the theoretical) of carefully prepared conductivity water may be regarded as due to H_2CO_3. Seyler and Lloyd (6) have shown that H_2CO_3 ionizes in two stages. They further found the ionization constant for the second stage to be $4{\cdot}27 \times 10^{-11}$ at 25°C. Wilkie (9) arrived at the value $3{\cdot}07 \times 10^{-7}$ for k_1, from conductivity measurements of carbonate solutions. As a source of hydrogen ions then, the second stage is negligible compared with the first, and for the purpose in hand H. HCO_3 may be regarded as a monobasic acid. The value of V obtained from equation 3 can be substituted in equation 2 in the usual way to calculate the degree of ionization of the H. HCO_3 and hence the pH of the water. Since V is expressed in c.c. units throughout, the value used for k_1 is $3{\cdot}07 \times 10^{-10}$. The value chosen for $\Lambda\infty$ H. HCO_3 was 353·2 at 18°C. obtained from D'Agostino and Quagliariello's value for the mobility of the HCO_3 ion and Kendall's (2) value for the hydrogen ion at 18°C.

The conductivity cells used for the measurements were similar to that described by Pennycuick (5). As a check on the calculated value for the pH attempts were made to measure the pH of water colorimetrically and by means of the hydrogen and quinhydrone electrodes.

Calculated and observed values are set out in Table 1.

The Quinhydrone Electrode.

The type of electrode vessel used and technique employed were similar to those employed by the author (7) when handling unbuffered solutions of high pH (4-5). The rubber stopper was supplied with two additional holes to carry an inlet and an outlet tube for maintaining an atmosphere of nitrogen. The quinhydrone was freshly prepared according to the method described by Valeur (8)

In eleven successive determinations the following values for the pH of water of $l = 0{\cdot}4 \times 10^{-6}$ mho were obtained: 5·8, 6·0, 5·7, 5·7, 5·9, 5·8, 6·0, 5·7, 5·7, 5·8, 6·0, giving a mean value of 5·8. This value is reasonably near the calculated value but its significance is rendered somewhat doubtful beecause of the variation between individual determinations as seen above and by the fact that at about pH 5·5 this electrode has reached its limit as a method of precision in unbuffered solutions, and is only qualitatively useful at a pH higher than this.

The reason is clear since Kolthoff and Bosch (4) pointed out that a saturated solution of quinhydrone in water would have a pH of 5·8, due to the ionization of the hydroquinone.

The Colorimetric method is therefore more likely to give the correct value. Although this method does not lend itself to fine distinctions, it can with the use of 'neutral' indicators be used to determine the pH of unbuffered solutions correct to one-tenth of a unit, and this suffices for the purpose in hand, namely,

a comparison with the calculated values. The indicator used was brom-cresol-purple.

As is seen from the table the values obtained by this method agree, within the limits of its accuracy, with the calculated values.

TABLE 1.

Specific conductivity mhos $\times 10^6$ at 18° C.	Molality of $H_2CO_3 \times 10^5$ (Calc).	pH (Calc).	pH measured colorimetrically.
0·30	·32	6·07	6·0
0·40	·53	5·95	6·0
0·50	·79	5·85	5·9
0·60	1·11	5·77	5·8
0·75 (author)	1·68	5·67	5·7 to 5·8
0·75 (Kolthoff)	1·55 (by titration)	5·7	5·7

The figures for water of specific conductivity, $0{\cdot}75 \times 10^{-6}$ mho agree very well with Kolthoff's both as regards CO_2 content and pH value.

The Hydrogen Electrode.

An attempt was made to measure the pH of water by means of a hydrogen electrode and is recorded as a matter of interest. The type of vessel used was that described in this Journal (Vol. V, 1928, 233). This electrode was coupled with a normal calomel electrode and the whole sunk in a thermostat at 30·0°C. A stream of hydrogen gas was sent through the water for fifteen minutes before introducing the electrode. The stream of hydrogen passing while readings are taken may be expected to displace more CO_2, continuously, and as a matter of fact the potentials drifted slowly towards a higher pH. The drift amounted to a tenth of a pH unit in about half an hour. Normally equilibrium is set up in five minutes, but owing to this drift an average value was taken. The usual precautions were taken to prevent contamination of the water, but these are not so effective when dealing with a bubbling hydrogen electrode as with a quinhydrone element. Values obtained in three representative determinations were 6·7, 6·8, and 7·05.

The pH of distilled water used for general laboratory purposes is usually taken to be 7. From the foregoing it is seen that even good conductivity water has a hydrogen ion concentration ten times as great as this. If not recognized this may lead to serious error when making up dilute solutions of acids or bases. The following example illustrates the care necessary when making up dilute solutions of bases.

With the object of preparing pure solutions of sodium hydroxide of strengths 1×10^{-6} N and 1×10^{-5} N respectively the author added the calculated amount of ·001 N sodium hydroxide solution to conductivity water ($1 = 0{\cdot}6 \times 10^{-6}$ mho) assuming the water to be near enough to neutral. The resulting solutions had reactions on the acid side of pH 7 in the first case, and

very nearly 7 in the second, instead of 8 and 9 pH units respectively. Reference to table 1 shows that in both cases a solution of sodium carbonate instead of the hydroxide would result, and that CO_2 was present initially in greater amount than the sodium hydroxide added.

The author wishes to thank Dr. S. W. Pennycuick for helpful suggestions.

REFERENCES.

1. D'Agostino and Quagliaviello: J.C.S. Abstracts, A 2 (1915) p. 426.
2. Kendall: See H. S. Taylor's "Treatise on Physical Chemistry," Vol. 1, p. 540.
3. Kolthoff: Biochem. Zeit., 168 (1926), p. 110.
4. Kolthoff and Bosch: Biochem. Zeit, 183, (1927), p. 435.
5. Pennycuick: Journ. Chem. Soc. (1927), p. 2602.
6. Seyler and Lloyd: Journ. Chem. Soc., 111 (1917), p. 138.
7. Thesis: University of Adelaide, 1927.
8. Valeur: Annal. de Chim. et de Phys., 21 (1900), p. 547.
9. Wilke: Zeit. Anorg. Chem., 119 (1921), p. 365.

ON THE NEUROFIBRIL STRUCTURE OF THE NERVE CELL (abstract)

by

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Since the discovery in specially stained nervous tissue of a complex system of fibrils within the nerve cell, considerable controversy has taken place concerning their nature and function: (a) Are they genuine cell constituents, or merely products of fixation of protoplasm? (b) Have they a conducting function? (c) Is there neurofibril continuity at the neurone junction?

(a) Against the existence of neurofibrils within the living nerve cell it has been urged that they can be seen only in cells whose protoplasm has been killed by fixation. The objection is not necessarily valid, since all methods of rendering such structures visible depend upon a difference in the optical properties of the fibrils and the surrounding neuroplasm, and an examination of fixed but unimpregnated tissue reveals that fixation alone is unable to render them visible. The excessive coarseness of the neurofibrils of some nerve cells distinguishes them from the fine fibrillary reticulum that one occasionally sees in fixed protoplasm. The statement that they can be seen only in fixed cells is moreover misleading, since they have been observed in living visual cells (Howard), in living ganglion cells of the leech, treated with very dilute methylene blue (Boeke), in some of the spinal cord cells of very young rabbits (personal observation), and in the nerve cells of Rhizostoma, where Bozler (4) finds that they can be seen, and even manipulated in the living cell.

(b) By asserting that the neurofibrils have a conducting function is not necessarily meant that they are the sole conducting structure of the nerve cell; we mean that they are intimately concerned with the process, in the sense that the magnitude of the transmitted disturbance is dependent upon the number of fibrils in a nerve fibre. (This conception does not, of course, infringe the "all-or-

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[1] These observations were carried out while holding a Rockefeller Travelling Fellowship in the Anatomy Department, Combridge, and in the laboratory of Histology and Embryology, Utrecht. To Professors J. T. Wilson and J. Boeke I desire to express my gratefulness for granting me the hospitality of their laboratories and for kindly interest in the work.

nothing'' law of conduction). It would be quite consistent with the view, for example, that conduction was the property of the neurofibril-neuroplasm interface.

The conducting function of the fibrils we can at present only infer from a consideration of their disposition within the cell; such considerations show that of the two possible functions, skeletal or conducting, the latter only can be inferred[2]. The evidence on which this conclusion is based is as follows:

(1) They occur in all nerve cells irrespective of whether they are in comparatively exposed regions (e.g., the gut plexuses) or are protected by bone (e.g., cells of cochlear nerve).

(2) They are found at a very early stage of embryonic development, e.g., two-day chick (Held); true skeletal structures develop much later.

(3) They run without a break along the axon from one end to the other; this is surely a fact of the deepest significance. It might be argued that in connective tissue cells a longitudinal arrangement of fibrils is seen. Their function in the latter case, however, is to oppose mechanical extension of the tissue, but only those axons that emerge from the cranium or spinal column could conceivably ever be subjected to extension, however slight.

The conclusion derived from a study of the axon is supported by examination of their arrangement in the body of the nerve cell. In cells of spinal ganglia where integration of conflicting impulses does not occur the fibrils have a simple arrangement; in the bipolar cells of fishes they pass directly through the cell, from one process to the other; in the ganglion cells of higher vertebrates where the bipolar condition is only temporary they enter the cell by the axon, coil as a bundle of varying complexity within the body of the cell and emerge again by the axon, the afferent and efferent neurofibrils separating at the T-shaped junction. It is difficult to see what function other than of conduction such an arrangement could subserve. (I do not think that there is any valid evidence for the statement often made that the impulse in spinal ganglion cells does not traverse the cell body. Retzius (8) likewise considers Bethe's Carcinus experiment invalid.) In the cells of the spinal cord on the other hand the fibrils converge from the dendrites upon the nuclear region of the cell, where they appear to form an anastomosing network, a small number of fibrils arising from this network to enter the axon. Here again the fibrils occupy the path that the impulses, reaching the cell via the dendrites, must take to enter the axon. As will be pointed out below, to make the evidence still stronger, in those cases where nerve terminals end upon the body of the cell (e.g., spinal cord cells, Mauthner's

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2 A third possibility, that they are nutritive structures conveying nourishment from the nuclear region to the most distant parts of the very long axons, seems to be invalidated by the fact that they are present in even the smallest nerve cells, e.g. the minute ganglion cells of the retina (Cajal) and even in the rod and cone cells (Howard). Why, moreover, are the intracellular neurofibrils in Mauthner's cell a direct continuation of the delicate nerve filament that ends on the cell?

cell, cells of tangential nucleus of trout) neurofibrils pass from the site of ending either directly to the cell axon, or indirectly via the perinuclear network. The latter condition is especially clearly seen in the leech. On the other hand, the statement frequently made that a single neurofibril may pass directly from one dendrite to another does not seem to be supported by careful observation. In my experience it has never been possible to trace a single fibril from dendrite to dendrite, unless that fibril has undergone branching within the body of the cell, in which case we are probably observing two fibrils entering the body of the cell from adjacent dendrites, and joining to form the network within the body of the cell.

(4) In the infundibular organ of Amphioxus Boeke has observed a direct connection between sense hair and intracellular fibril.

(5) The early degeneration of neurofibrils in ruptured nerve trunks is difficult to reconcile with the view that they are inert skeletal structures. In senile dementia Bielschowsky observes extensive neurofibril degeneration.

(6) Bozler (4), working with the nerve cells of Rhizostoma, where the fibrils can be seen in the living cell, finds that they are plastic to mechanical manipulation, and devoid of properties that one would associate with skeletal structures.

From these considerations it seems difficult to regard the neurofibrils as anything but conducting elements.

(c) *Is there neurofibril continuity at the neurone junction?* The theory of neurone discontinuity arose in the observations of Forel that in Golgi preparations the fine branching collaterals ended blindly. The evidence is perhaps of little value to-day, since it can be shown that the Golgi impregnation may give but an incomplete impregnation of the finer nerve fibres. Furthermore, the blindly ending "Endbäumchen" are almost certainly not identical with the supposed freely ending terminations that the improved reduced silver processes reveal. By these newer methods it has been found possible to examine the Held-Auerbach end-feet in some detail; nevertheless, even with these processes it is so difficult to obtain sharply-defined results that competent observers, examining the same material, have come to opposite conclusions. By Cajal it is held that the fine nerve terminals come into close association with the surface of the nerve cell, but do not fuse with it, a sharp line of separation being seen between the two elements (discontinuity). This view is opposed by Held, Bielschowsky and Wolff, and others, who find that such a separation does not occur, and that fibrils from the terminals may become visibly continuous with those of the nerve cell (continuity).

It is frequently stated that physiological studies of the reflex decisively support the theory of discontinuity. Such criticism is invalid. It is, of course, quite impossible to avoid the conclusion that ultimately there must be some form of discontinuity at or near the region of integration. Such a "discontinuity"

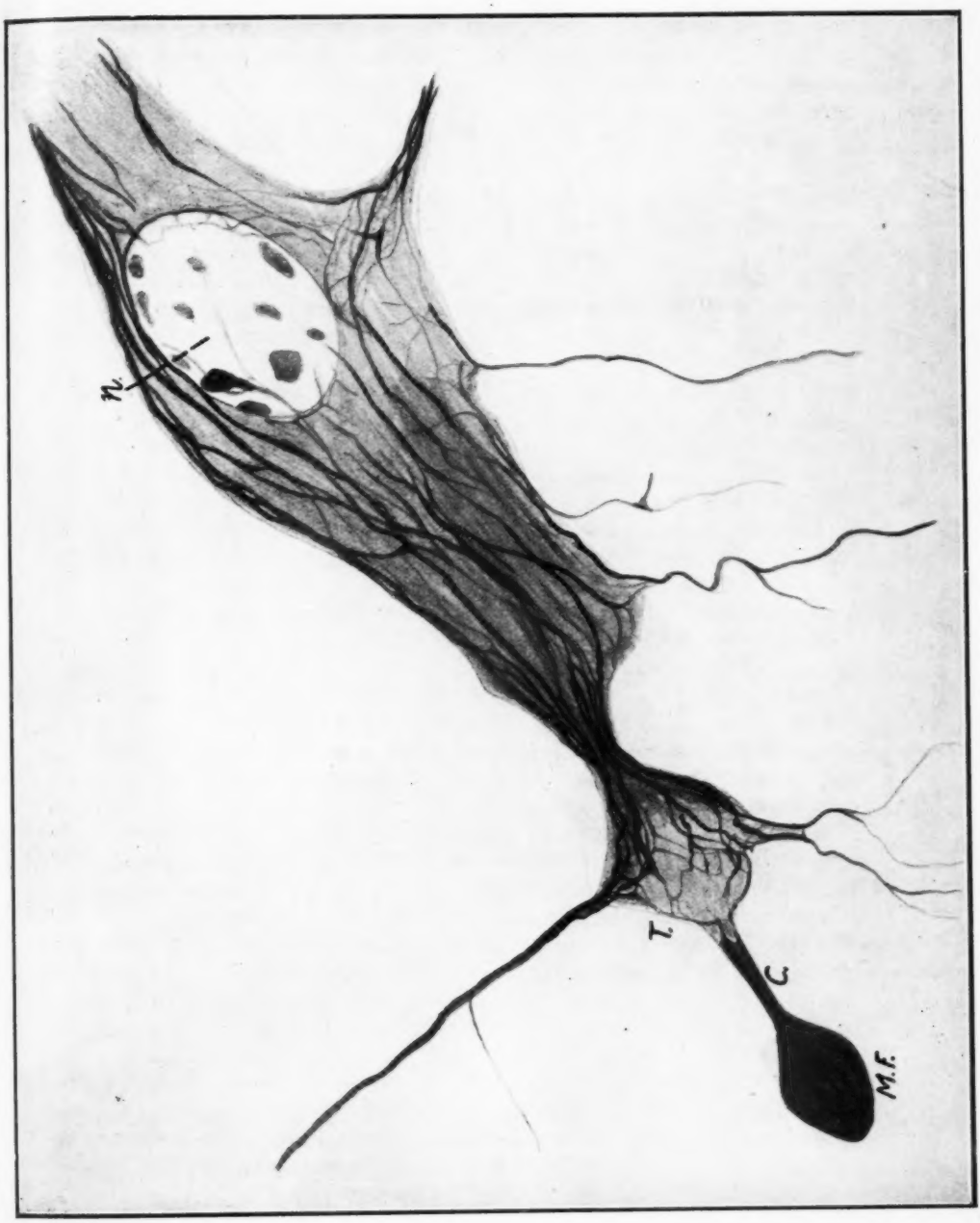
may be in the form of a change in the nature of the conducting protoplasm, or due to the presence of a transverse membrane with special physical properties, at some point along the fibrils, either at the surface of the cell or in its interior; but it is surely not valid to argue that the discontinuity must be in the form of a visible gap in the conducting chain at the surface of the cell. Close examination of the nerve terminals shows that such a gap seems to occur only as an artefact. The following terminations have been investigated:

(1) *Nerve terminations on Mauthner's cell.* Mauthner's cells are a pair of very large cells situated in the medulla oblongata of fishes and tailed amphibians; on them terminate fibres from the vestibular nerve and its nuclei, tecto-bulbar fibres, and fibres from the acoustico-lateral nuclei. In Bielschowsky preparations the nerve cell is seen surrounded by a mass of thick axons, which, if the section has been suitably cut, are observed to taper off into a delicate thread, visible under only high magnification. This delicate terminal approaches the surface of the nerve cell. In the majority of preparations the impregnation of intracellular fibrils fails (except at the base of the dendrites); in such cases the terminals end short at the surface of the cell (discontinuity). Only seldom is it possible to obtain a general impregnation of the neurofibrils throughout the body of the cell. Examination of such cases shows (though great care must be taken to observe it accurately) that the neurofibrils are a direct continuation of the neurofibril of the nerve terminal. My observations therefore confirm those of Marui (6), and are contrary to those of Bartelmez (1). The latter author describes the termination of very coarse fibres on the lateral dendrite of Mauthner's cell. The endings are held to come into close contact with the surface of the dendrite, but, nevertheless, to afford an admirable instance of discontinuity, since with good fixation they actually exhibit a plasma membrane at their free ends. In my preparations I can find no trace of such endings; I find, on the contrary, a plexus of very fine fibres on the surface of the dendrite, ending here by means of typical end-feet. If Bartelmez figure in support of his conclusions be examined (figure 13 of his paper) it will be seen that the supposed nerve terminals all end on what is obviously the plane of the microtome section (cut edge of dendrite). They appear to me to be merely the cut ends of the thick axons that run beside the lateral dendrite. Such axons, I find, may present swellings along their length, which may account for the variously swollen endings in Bartelmez' figure.

(2) *Connection between collaterals of Mauthner's fibre and cells of spinal cord (Amblystoma).* This connection offers a unique opportunity for studying

EXPLANATION OF PLATE.

Fig. 1. The junction between a collateral of a giant Mauthner fibre and large nerve cell of spinal cord of Amblystoma. (Bielschowsky preparation. Zeiss Apochromat. 1.5 mm. oc. × 15.) M.F., Mauthner's fibre in transverse section; C., collateral of Mauthner's fibre; T., "transitional tissue"; N., nucleus of nerve cell.


n.
T.
C.
M.F.

the structure of neurone junctions. In transverse sections of the cord the two Mauthner's fibres are easily recognizable, even with low magnifications, in the ventral white matter. At intervals they give off very short truncated collaterals (see figure 1), which communicate with the dendrite of the large spinal cord cells. They may communicate with one, two, or even three dendrites, and may be associated occasionally with even more than one nerve cell. Sometimes the dendrites are long and tenuous, at other times short and thick, and, of course, intermediate gradations exist. Now occasionally a distinct break occurs between the swollen ends of the collateral and the dendrite (discontinuity). But this is merely due to imperfect impregnation, for in all other cases a feebly staining protoplasmic strand passes from the ending and merges insensibly into the protoplasm of the nerve cell. On this point I can confirm the observation of Beccari (2), contrary to Bartelmez. But I find in the best impregnated specimens that this very weakly staining "transitional tissue" is traversed by exceedingly delicate fibrils, which undergo a gradual transition into the coarse fibrils of the nerve cell, and, in fact, connect these fibrils with those of the nerve terminal. It is clearly not possible to demonstrate any gap in the protoplasm.

(3) *Spinal Cord Cells.* Although these cells are the most commonly used in the study of the structure of neurone junctions, they are, in my experience, ill adapted for that purpose. In Cajal and Bielschowsky preparations of the cords of young rabbits no trace of endings of fibres on the cell body is to be observed; perhaps our methods cannot reveal them, but one can certainly see no trace of them. As previously reported (10), the neurofibrils enter the body of the cell entirely through its dendrites. As we trace them away from the cell we find that they gradually separate outwards in the branches of the dendrites, but it is never possible to find an ending for them. Similarly the branching collaterals do not exhibit any true ending, in my experience. From this I conclude that in these young cords the neurofibril of the collateral is continuous with the neurofibril of the dendrite. The ending of the collateral of Mauthner's fibre, above described, is merely a special case of such a junction. As previously described, it is exceedingly difficult to obtain *direct* evidence for such a mode of union in spinal cord tissue. The cells of the cerebral cortex are better adapted to this purpose, for the cells of the cortex may receive endings not only from the thalamus and from distant parts of the cortex, but from closely adjacent nerve cells. In Golgi preparations of the cat's cerebral cortex the axon, readily identifiable, is seen to give off shortly beyond its origin numerous very delicate collaterals, that undergo but little branching; in appearance they are identical with fine branches that appear to grow out from the dendrites of the nerve cell. When traced individually they are found usually to emerge from the plane of the section, or to end in a gradually tapering filament, or in a large varicosity, in relation to nothing. It is unjustifiable to regard these endings as true endings in close contact with unstained nerve cells. On the contrary, in favourable cases

one may observe that the fine collateral is continuous with, and is, in fact, one and the same thing as the delicate branch that appears to grow out from the dendrite.

In my previous observations (10) I could find no evidence for the existence of end-feet on the surface of the cell. My conclusion that they did not, in fact, occur was erroneous, for it applies only to the young animal. In adult animals they can usually be well exhibited in Bielschowsky material; it is only from young material that they appear to be absent. As the animal increases in age the structure of the cord is found to increase enormously in complexity. It would appear that the later developed fibres terminate, in part at any rate, in end-feet. By Cajal they are stated to lie in close contact with the nerve cell. The very important observation has been made by M. Heidenhain (5) that they traverse the shrinkage space that usually occurs around the nerve cells. It follows, therefore, that the endings cannot possibly be free, as frequently stated, but must be attached to the surface of the cell. Opinion may differ as to whether they are "glued" to the cell, or are in protoplasmic continuity with it; but it is not possible to speak of "free" endings in the usual sense of the word. Bielschowsky confirms this observation (3), but finds further that fibrils pass from the ending into the nerve cell (continuity). This is denied by Heidenhain.

In Bielschowsky preparations of the cord of the adult cat I find that Heidenhain's observation of fibres traversing the shrinkage space may readily be confirmed. Many of them may tear away during the process. But great difficulties are encountered when we try to make exact observations on the passage of fibrils into the cells. For the fibrils of the adult cell are so delicate (in contrast to those of the young animal), and they stain so weakly, that exact observation is seldom possible. Many cases exhibit no trace of passage of fibrils into the cell (compare Mauthner's collateral above described), but in comparatively few cases, where the impregnation has been very sharp, the neurofibril continuity can be observed. Such fibrils are found to pass into the substance of the cell, and not merely lie on its surface. Frequently there may be seen between the ending and the surface of the cell an ill-defined granular substance. It is usually held to be inert adhesive "Kittsubstanz." It may here be suggested that it is in reality similar in nature to the "transitional tissue" so well seen in the junction between Mauthner's collateral and spinal cord cells in Amblystoma. The Held-Auerbach end-feet occur only on the body and proximal part of the dendrite of the cell; the extensive dendritic ramification is in my experience devoid of them.

(4) *Cells of Sympathetic System.* The observations on cells of young animals it was found possible to extend to the sympathetic system (11); i.e., the collaterals of the preganglionic neurones are directly continuous with the dendrites of the sympathetic cells. Stöhr has independently come to a similar conclusion (9). True pericellular endings embracing the body of the cell can

be demonstrated in the sympathetic ganglia of the frog, but it has not been possible to determine whether neurofibril continuity occurs at this junction.

(5) *Cells of Trapezoid Nucleus.* The very characteristic calyces of Held have been the subject of investigation, subsequently, by Cajal and by Bielschowsky. According to Cajal they clasp the cell and constitute an admirable example of contact. Bielschowsky (3) finds that such contact is only observed when the impregnation of intracellular fibrillae is incomplete; as soon as these fibrils impregnate their connection with the intracellular fibril is recognizable.

My own observations confirm those of Bielschowsky. When the intracellular fibrils fail to stain, one undoubtedly has the appearance of a clasping nerve terminal closely embracing the body of the cell. But when the fibrils impregnate sufficiently sharply to be clearly visible, one may observe many cells where the fibrillar mass is so dense that it would be quite impossible any longer to maintain that the two fibril systems were distinct. Such preparations as a rule are unsuitable for deciding this point, since it is practically impossible to determine with precision whether the intracellular fibrillae are connected with the terminal or merely pass closely underneath it. But when cells are selected where the intracellular fibril system is less developed, i.e., when the fibrillae may be observed individually, one quite commonly observes cases where critical observation is possible, especially if cases are selected where the nerve terminals are examined in strict profile. In such cases I find, in confirmation of Bielschowsky, that the more weakly impregnated intracellular neurofibrils are in direct connection with those of the clasping terminal.

(6) *Tangential Nucleus (Tello) of Trout.* Coarse neurones of the eighth nerve, on their way towards Deiters' nucleus, establish connection with the rounded cells of the tangential nucleus, at the entrance of the eighth nerve into the brain. The actual connection is established by a very short outgrowth from the eighth root fibre, which is applied by a broad expanse to the surface of the rounded cells of the tangential nucleus. When the impregnation is sufficiently sharp to render the intracellular fibrillae moderately clearly visible, the passage of numerous fibrils of the utmost delicacy from the terminal into the nerve cell may be observed. They do not even appear, so far as it is possible to observe, to form a perinuclear net, but pass directly into the axon.

(7) *Cells of Purkinje.* The investigation of these cells with present methods has proved a matter of very great difficulty. That no connection between intracellular fibrillae and the extracellular terminals can be observed in almost every case cannot be denied. But such cases are usually quite useless for deciding the question, since in almost every case the intracellular fibrils are either invisible or so faint that it is not possible to make reliable observations on them.

Concerning the clasping terminals of the basket cells, that embrace the body of the nerve cells, I can state in confirmation of Oudendal (7), that when the delicate intracellular fibril meshwork is rendered sufficiently clearly visible, and

if cases are selected where the branches of the pericellular ending are viewed in true profile, the passage of delicate fibrils from the branching ending into the intracellular meshwork can be observed; the fibrils arise usually, but not always, from minute thorn-like processes from the inner surface of the clasping terminal. But no indication of such connection has been observed between the climbing tendril fibres that spread over the greater part of the dendritic development of the cell. It seems probable that we have here a true instance of a fibre ending upon a nerve cell by contact, and offering an instance of a truly discontinuous synapse. But further investigation is necessary to establish its validity. At the same time it would seem quite clear that an ending of this type, which spreads over such a great area of the cell surface, must have some different mode of action from that of a nerve terminal (such as we see, for example, in the nerve endings on Mauthner's cell above described), where contact, if it were contact, would be established by only the tiniest speck of protoplasm.

CONCLUSIONS.

1. The neurofibrillae are true cell constituents, and not products of fixation.

2. They are intimately concerned with conduction; this conclusion is an inference from the fact that they occupy the path that the nervous impulses traverse.

3. There is no visible gap in the neurofibril system at the neurone junction; the climbing tendril fibres of the cerebellar cortex seem to be an exception to this rule.

REFERENCES.

1. Bartelmez, G. W.: Journ. Comp. Neurol., 25 (1915), p. 87.
2. Beccari, N.: Arch. Ital. di Anat. e di Emb., 17 (1918), p. 239.
3. Bielschowsky, M., in Lewandowsky's Handbuch der Neurologie, vol. 1 (1910), and in Möllendorf's Handbuch d. mikr. Anat. des Menschen, (1928).
4. Bozler, E.: Zeit. f. vergl. Physiol., 6 (1927), p. 255.
5. Heidenhain, M.: Plasma und Zelle (1911).
6. Marui, K.: Journ. Comp. Neurol., 30 (1918), p. 127.
7. Oudendal, A. J. F.: Psychiatrische en Neurologische Bladen (1912), p. 1.
8. Retzius, G.: Proc. Roy. Soc. London, B. 80 (1908), p. 414.
9. Stöhr, Ph. (Jr.): Zeit. f. Zellforschung u. mikr. Anat., 5 (1927), p. 117.
10. Tiegs, O. W.: Aust. Journ. Exp. Biol. and Med. Sci., 4 (1927), p. 25.
11. Tiegs, O. W.: Aust. Journ. Exp. Biol. and Med. Sci., 4 (1927), p. 79.

A STUDY OF CALCIUM METABOLISM IN THE HUMAN FEMALE

by

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In the course of investigations into the hormonal control of sex function in the human female, estimations of blood calcium during the menstrual cycle and in pregnancy have been made.

The data here presented were complete in November, 1928, but as it was hoped to complete a wider correlation publication was delayed.

The first observer to investigate blood calcium during the menstrual cycle was Blair Bell (1), who published his findings in 1908. Bell, however, did not regard the alteration of the calcium as being of hormonic origin, but rather regarded the loss of calcium as analogous to the laying of an egg by a bird; he showed, in fact, that hens had lower blood calcium after laying an egg, and the results obtained in these investigations show that the drop begins just prior to menstruation, and continues to fall during that period.

Considerable work has been done on the blood calcium during pregnancy by Widdows (2), while the influence of diet on blood calcium was investigated by Dennis and Minot (3), and the result of injecting calcium into the blood stream has been discussed in detail by Clark (4).

In 1924 Cook (5) published his findings concerning Blood Calcium and Toxaemias of Pregnancy, but was unable to find any correlation between the two.

The report of the work of Widdows was to the effect that there is a slight increase of blood calcium in the earlier months of pregnancy, but at a later period the amount is considerably reduced. This latter was thought to be due to the foetal requirements making inroads on the mobilized calcium. Watchorn (6), in 1924, published the blood calcium estimations in pathological conditions, and two years later she (7) gave the results of blood calcium investigation during pregnancy, but attention was directed mainly towards the serum magnesium.[1]

[1] Close and Osman have published the results of their investigations of the blood calcium of women during the menstrual cycle, and they conclude that there is no variation (13). From the curves herein depicted it is seen that the blood calcium variations do not occur at the midway or midmenstrual points as taken by these authors, but only by the serial investigations do these figures offer a true index of the calcium metabolism during the menstrual cycle.

The year following this work Mirvish and Bosman (8) correlated the blood calcium content with the secretion of the ovarian hormone, and then demonstrated that the introduction of follicular extract (prepared according to the method of Allen and Doisy (10) into rabbits reduced the blood calcium. The same result was also found when the extract was injected into humans (9), either male or female.

The present work covers a period of nearly three years, during which the blood calcium of over seventy different patients has been estimated in the varying phases of their menstruation. The blood of these patients could only be taken when they reported for treatment on certain specified days. The result of this has been that on several occasions the patient's blood has been taken in precisely the same phase, i.e., in relation to the menstrual flow, but these results agreed very nicely, thus providing an unconscious check upon the analyses, while the blood calcium estimated during other phases of the cycle differed appreciably from these values.

As an instance of this type of case, we have the following:

			Blood calcium.
Alice M., æt. 28 yrs.	Blood taken 29/7/25, two days	before menstruation	11.5 mg. per 100 cc.
" " "	20/12/27, three	" "	11.6 " "
" " "	10/7/28, two	" "	11.5 " "

The same case, however, taken four days after the onset of menstruation had blood calcium 10·3 mg. on 14/8/28, and on the fifth day which was 17/7/28, her estimated blood calcium was 10·5 mg. per 100 cc. of blood. Twelve days after the onset, 24/7/28, the amount was 11·0 mg. per 100 cc.

For comparative purposes the results in some cases are graphically expressed. Generally it is found that the highest point of the curve is about five days before the onset of menstruation. From this point the blood calcium content rapidly falls, until about the third day of menstruation. After this there is a very slight fall until about the seventh day after menstruation, when the blood calcium gradually rises again to the acme about five days before menstruation. The variations are not as a rule very marked, but average between 9·5 mg. per 100 cc. of blood to 11·5 mg. or perhaps a little more. A statistical comparison of pre-menstrual blood calcium values with corresponding menstrual and post-menstrual results has been possible in a series of eight determinations. In the other cases, owing to the fact that the material was subject to ordinary clinical availability, such an analysis was impossible. Some idea of the accuracy of the estimations and their value as a basis for deduction can be gained from an analysis of these eight cases:

Average difference between pre-menstrual and menstrual blood calcium values ..	= 7.7%
Mean error in the series	= ± 5.8%
Average difference between pre-menstrual and post-menstrual blood calcium values	= 13.8%
Mean error in the series	= ± 8.2%

Thus it can be seen that though the error is high it is definitely less than the amount to be measured, especially in the latter instance. The error is a combination of (a) accidental variations among the subjects, (b) accidental errors in calcium estimations. It can be safely asserted on the above findings that there is a difference of at least 5% between the blood calcium of the pre- and post-menstrual phases. When it is remembered that Kramer and Tisdall's method of calcium estimation is somewhat elaborate, and that the analyses in these cases were carried out during routine work at the Adelaide Hospital, the results are not unsatisfactory. Slight variations, such as the time of centrifuging or the rate of same, would lead to errors quite as large as those indicated. The present results offer the only conclusive evidence yet adduced for blood calcium variation in the normal menstruating female.

The cases of pregnancy gave results which entirely conform to those of Widdows, with the exception of a slight rise in these cases during the last two months. The average of three sets of cases taken at different times during parturition, were:

1st month.	Between 2nd and 5th month.	5th to 7th months.	7th to 9th months.
11·3 mg.	10·8 mg.	9·41 mg.	9·87 mg.

Blood drained from placenta contained 10·51 mg. per 100 cc. of blood.

Having in mind the results of Mirvish and Bosman, it is thought that the reduction of blood calcium in the later months of pregnancy is not entirely due to foetal requirements, but it is considered that the calcium is reduced as the ovarian hormone increases. This increase has been indicated in the work on mice, where the serum of pregnant women is injected and the ratio between total mouse weight and uterine weight is estimated and the amount of hormone guaged according to the ratio. Frank and Goldberger (11) noticed also that the maximum ovarian hormone existed immediately before menstruation.

Ascheim and Zondek (12) have indicated that the amount of follicular hormone increases as pregnancy progresses. This intimation accords with the findings of a reduction of the blood calcium content the further the patient advances towards full term and lends further support to the contentions of Mirvish and Bosman (9 and 10).

Protocol.

Subject.	Pre-menstrual Phase.		Menstrual Phase.		Post-menstrual Phase.	
H.S.	14·0 (4)	14·0 (1)	10·1 (2)	10·35 (3)		11·6 (10)
M.S.		14·46 (1)	10·2 (2)		9·5 (2)	
C.Q.	12·1 (3)	10·1 (1)	10·42 (1)	10·6 (2)	10·5 (1)	10·4 (10)
D.C.	12·5 (10)	13·1 (4)	12·4 (3)			11·2 (8)
A.M.	11·6 (3)	11·5 (2)	10·3 (4)			11·0 (7)
	11·5 (1)					
M.B.	11·07 (8)	11·4 (7)	10·3 (2)			
	11·0 (6)					

Subject.	Pre-menstrual Phase.		Menstrual Phase.		Post-menstrual Phase.	
L.S.	11.56 (5)		11.3 (2)	10.0 (4)	10.2 (1)	9.2 (4)
H.D.	11.4 (5)	11.01 (4)	10.9 (4)			
L.W.	12.6 (2)		10.22 (4)			11.2 (7)
C.B.	10.4 (10)	11.3 (6)	10.9 (2)	10.8 (4)	10.8 (2)	10.8 (3)
M.D.		11.4 (4)		10.2 (4)		11.4 (10)
P.N.		11.0 (6)	11.6 (1)		10.4 (2)	
I.E.		11.23 (2)				9.75 (8)
B.H.			10.6 (4)		9.0 (3)	
B.C.						11.2 (7)
N.B.			10.2 (4)		9.2 (4)	
L.S.		11.3 (2)	10.2 (1)		10.0 (3)	
M.J.					10.1 (2)	
M.P.		10.9 (4)	9.5 (1)			
N.W.					10.4 (5)	
R.S.	12.8 (10)		11.6 (1)			14.5 (10)
R.W.		11.7 (5)			11.2 (4)	
A.N.		11.6 (5)			11.0 (5)	
V.B.	11.7 (7)					
A.S.			11.2 (4)			
N.D.	11.4 (12)		10.2 (4)			
E.P.					10.6 (6)	
E.C.			10.6 (1)		10.0 (7)	10.1 (10)
M.C.					11.0 (7)	
F.G.					10.7 (7)	
K.H.	10.8 (11)					
I.S.	12.1 (6)		10.3 (3)			
M.B.	11.1 (4)					10.7 (9)
E.S.	12.4 (4)				11.2 (7)	
S.H.					10.9 (3)	
M.K.	11.4 (5)		10.8 (2)			
A.F.			10.4 (2)			11.2 (8)
I.K.	11.3 (7)		10.2 (3)			11.1 (7)
A.W.			11.2 (4)			
L.B.						9.9 (9)
E.G.	10.5 (12)	10.4 (1)	9.8 (4)			

In the above protocol the menstrual cycle has been divided into three phases for convenience; the menstrual phase consists of four days, while both pre-menstrual and post-menstrual phases comprise twelve days each, thus making a total of twenty-eight days for the complete cycle. The initials of the subject are given on the left-hand side, and the number of milligrammes per 100 cc. of blood removed from the subject are placed in the column according to the phase of the menstrual cycle when it was taken. The bracketed numeral after the calcium figure denotes the number of days of the phase either before, after the onset, or after the conclusion of menstruation.

My thanks are due to Professor C. Stanton Hicks in whose department and under whose supervision these researches were conducted, and to Mr. M. L. Mitchell for his help in the estimation of calcium in the blood of pregnant women, and to Miss M. Long, Adelaide Hospital.

SUMMARY.

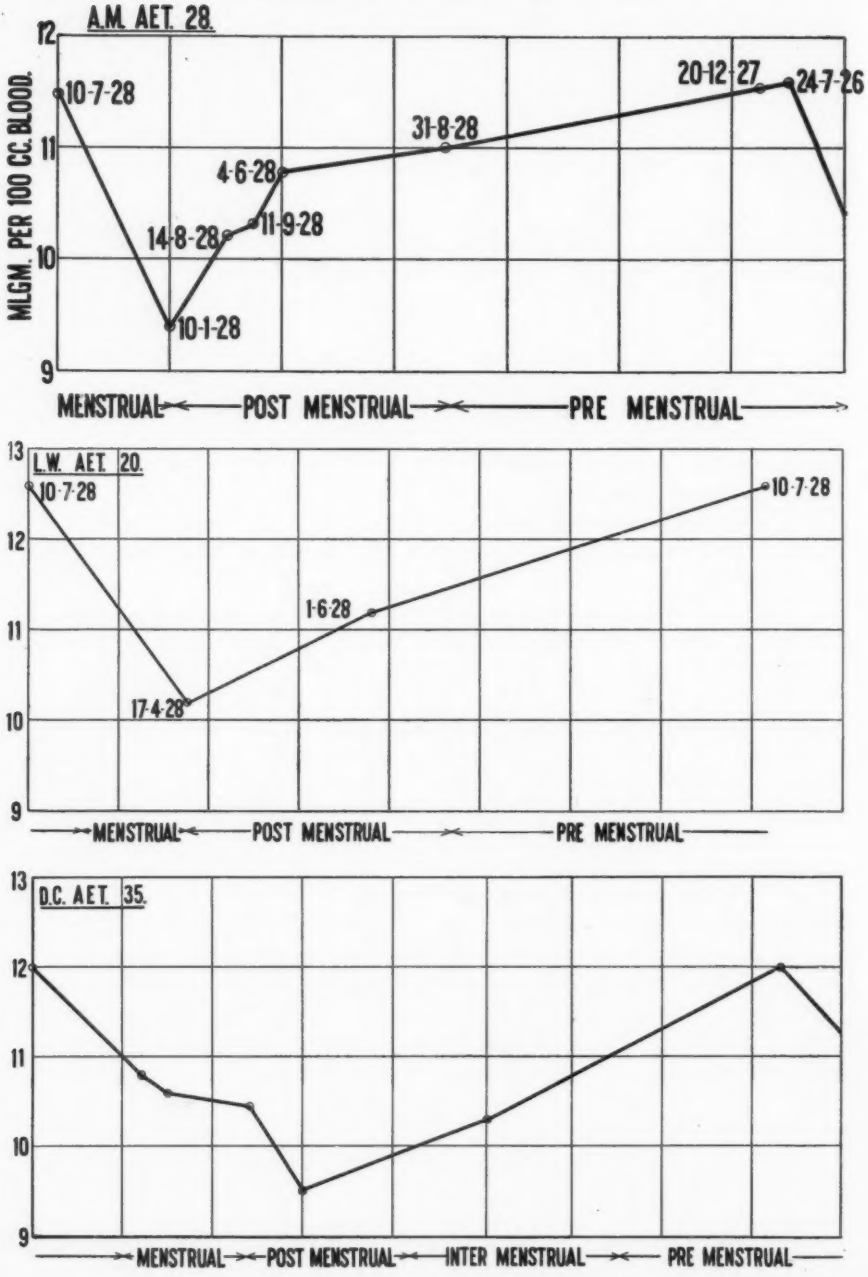
An examination of blood calcium during the menstrual cycle has been made in 80 cases presenting themselves clinically. In 30 cases enough consecutive data

have been accumulated to enable conclusions to be drawn. The blood calcium shows a pre-menstrual rise followed by a fall beginning just prior to the onset of the menstrual flow, the variation being not less than 5%, and probably nearer 8%, of the normal calcium level.

Suggestions for previous failure to detect such alteration are given.

BIBLIOGRAPHY.

1. Blair Bell, W.: Menstruation and its Relationship to the Calcium Metabolism. Proc. Roy. Soc. Med., pt. I (1908), p. 291.
2. Widdows, S. T.: Calcium Control of the Blood during Pregnancy. Biochem. Journ. 75 (1924), p. 555.
3. Dennis, W. and Minot, S.: Effect of Feeding with Calcium Salts on Blood Calcium Content. Journ. of Biol. Chem., 41 (1920), p. 357.
4. Clark, G. W.: Effects of Hypodermic and Oral Administration of Calcium Salts on the Calcium Content of Rabbit Blood. Journ. Biol. Chem. 43 (1920), p. 89.
5. Cook, Frank: Observations on the "Toxaemias" of Pregnancy. Guy's Hosp. Rep. 74 (1924), p. 172.
6. Watchorn, Elsie: Calcium and Magnesium in Pathological Sera. Quart. Journ. Med., 18 (1924), p. 288.
7. Watchorn, Elsie: Magnesium and Calcium in Blood during Menstrual Cycle. Brit. Journ. Exp. Par., 7 (1926), p. 120.
8. Mirvish, L. and Bosman, L. P.: Influence of the Internal Secretions of the Ovary on the Calcium Blood-level and on Calcium Metabolism. Quart. Journ. Exper. Physiol., 18 (1927), p. 11.
9. Mirvish, L. and Bosman, L. P.: Effect of Ovarian Extracts on the Calcium Blood-level in Man. *Ibid.* (1927), p. 29.
10. Ellen, E. and Doisy, E. A.: An Ovarian Hormone. Journ. Amer. Med. Assoc., 71 (1923), p. 819.
11. Frank, R. T. and Goldberger, M. A.: The Female Sex Hormone. Journ. Amer. Med. Assoc., 86 (1926), p. 1687.
12. Ascheim, S. and Zondek, B.: Schwangeschaftsdiagnose aus dem Harn durch Hormonnachwiess. Klin. Wochenschr., 7 (1928), p. 8.
13. Close, H. G. and Osman, A. A.: Biochem. Journ., 22, No. 6 (1928), p. 1544.


A.M. AET. 28.
MLGM. PER 100 CC. BLOOD.
12
11
10
9
10-7-28
10-1-28
14-8-28
4-6-28
11-9-28
31-8-28
20-12-27
24-7-26
MENSTRUAL
POST MENSTRUAL
PRE MENSTRUAL
L.W. AET. 20.
13
12
11
10
9
10-7-28
17-4-28
1-6-28
10-7-28
MENSTRUAL
POST MENSTRUAL
PRE MENSTRUAL
D.C. AET. 35.
13
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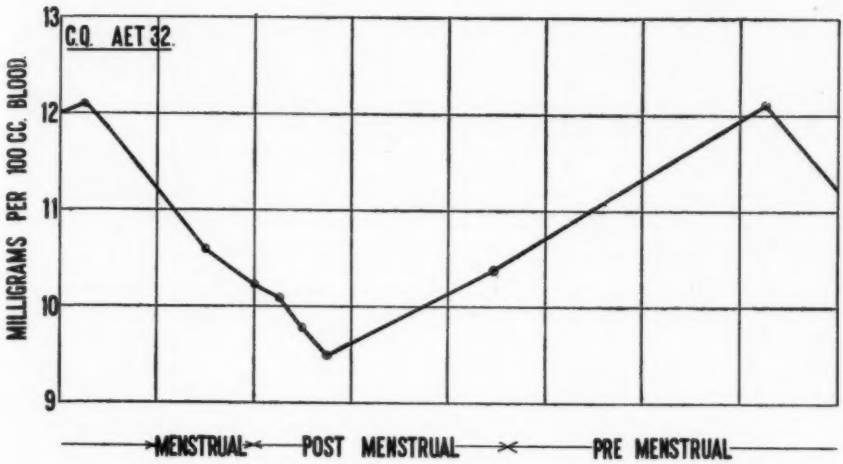
MENSTRUAL
POST MENSTRUAL
INTER MENSTRUAL
PRE MENSTRUAL


C.Q. AET 32.
MILLIGRAMS PER 100 CC. BLOOD
13
12
11
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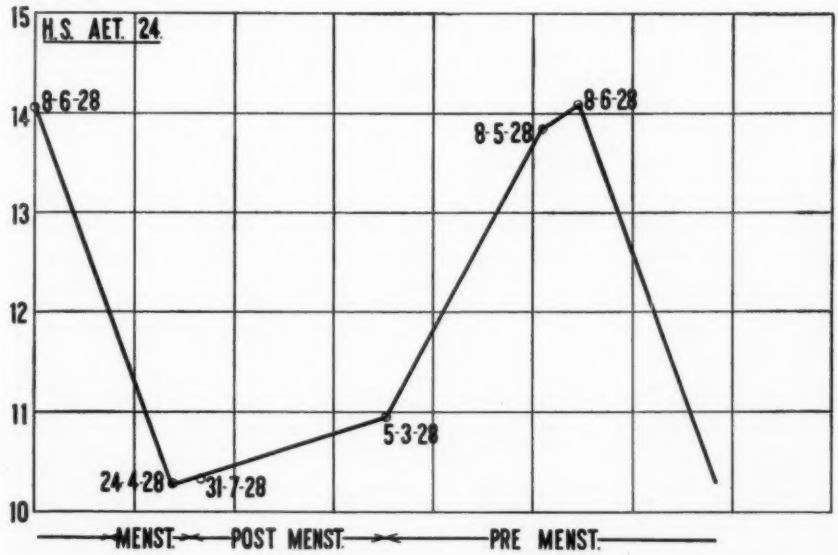
9
MENSTRUAL
POST MENSTRUAL
PRE MENSTRUAL


H.S. AET. 24.
15
14
13
12
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8-6-28
24-4-28
31-7-28
5-3-28
8-5-28
8-6-28
MENST.
POST MENST.
PRE MENST.

THE RELATION BETWEEN WATER CONTENT AND AMOUNT OF PHOTOSYNTHESIS

by

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(Submitted for publication 8th May, 1929.)

The present paper describes some preliminary work in an investigation concerned with the operation of "time factors" in photosynthesis. The work includes the changes in the amount of carbon assimilation with the age of the leaves and the effect of differing light intensities and of different temperatures on leaves of different ages and at different times. It will be described in detail in other papers.

In work of the above nature it is obviously impossible to use the same experimental material, and different leaves must be used for different experiments. Therefore one of the first experimental procedures is to determine the variability in amount of photosynthesis per standard area in leaves of the same age. In particular, the possibility of the amount of water present in the leaves affecting the rate of assimilation had to be considered. This was necessary for three reasons: first, the possibility of direct proportionality between water content and photosynthesis; secondly, the fact that when leaves are first placed in the apparatus used the rate of assimilation reaches a maximum, and then attains a lower steady value, with a possibility that this is due to changes in water content; thirdly, the fact that Dastur (1) has recently stated that the fall in rate of photosynthesis with the onset of senescence is due to changes in the water content of the leaf.

The leaves used were those of the Cherry Laurel (*Prunus laurocerasus*, L.), and were all picked from similar positions on the shoots, namely, the fourth leaf from the top of each shoot.

The apparatus was of the type first designed by Dr. F. F. Blackman, details of which and of its sources of error will be published at a later date. The leaves used for the experiments are placed in a chamber in the apparatus, and the whole of the closed apparatus filled with hydrogen and a known volume of carbon dioxide. These gases are constantly circulated by means of a pump, and the oxygen formed on illumination of the leaf passes, together with the hydrogen, through a tube containing palladium black. This catalyzes the union of hydrogen

and oxygen forming water. The diminution in volume of the gases in the apparatus is read by means of a gas burette, which forms part of the apparatus, and such diminution in volume is equal to three times the amount of carbon dioxide utilized in carbon assimilation. Readings of the burette are made at definite intervals, and the difference between any two readings is taken as the amount of assimilation performed during that period.

It has been observed for a long time that gross changes in the water content of leaves cause a decrease in the amount of photosynthesis (Kreusler, 4; Déhérain and Maquenne, 2). Systematic observations were made by Thoday (5), and still later ones by Iljin (3). The latter observers found that the rate of photosynthesis was depressed on wilting, and that even before plasmolysis occurred this was the case. This they ascribed to changes in the size of the aperture of the stomates, whose movements they followed by means of a porometer. This correspondence of rate and water content referred to visible changes in the leaf, as is evident from their terms, "turgid," "flaccid," "limp," etc.

Recently, however, a paper has appeared by Dastur (1), in which it is stated that "The results obtained indicate a closer relationship between the rate of assimilation and the water content of the leaf. With decrease in the rate of assimilation there is a corresponding decrease in the water content of the leaf. The reactions between the two are so close that when the rates of assimilation of the leaves of any plant are plotted against corresponding values of the water content the points lie very nearly in a straight line." There are several criticisms to be levelled against Dastur's technique; the chief is the fact that different leaves at different stages of their development were used in order to obtain varying water contents. Also the rates of photosynthesis were measured by means of absorbing in soda-lime tubes the amount of carbon dioxide not utilized by the leaves on illumination, and then weighing the tubes. Atmospheric air was used, and the differences in the amount of CO_2 to be weighed were usually tenths of 1 milligramme. Apart from any "biological variation" in the leaves, it is incorrect to assume that changes due to age are due to water content alone, and further stomatal openings must have played an important part in determining the amount of photosynthesis where such minute amounts of carbon dioxide are concerned. On *a priori* grounds, and judging from an extensive experience with cherry laurel leaves, it does not seem feasible, for example, that a variation in water content from 1·110 grammes to 1·043 grammes in two different normal leaves from the same plant should reduce the amount of assimilation from 0·0013 grammes CO_2 to 0·00018 grammes CO_2 per hour per 100 sq. cms. of leaf area. We present, however, our own data below, and this shows that there is a fairly wide range of water content of the leaf over which the rate of carbon assimilation remains constant.

In all cases the amount of CO_2 used was high (about 8%), so that the possibility of a decrease in rate due to stomatal movements is minimized, for

with large amounts of CO_2 Brown and Escombe have shown that variations in the amount of CO_2 to enter the stomates only occurs when the stomate is relatively tightly closed. Two intensities of light were used in these experiments, one at 8,160 lux (metre-candles), and one at 27,500 lux. The temperature throughout was 16·0°C., and the amount of assimilation is expressed by 25 sq. cms. of leaf area.

In Tables 1 and 2 are given the amounts of assimilation per hour of different leaves, and also the water content of the corresponding leaves.

TABLE 1.

Leaves picked directly from the bush. Temperature, 16° C. Light intensity, 8,160 Lux. CO_2 content, 8·0%.

Leaf.	Amt. of water (grms.).	Assimilation per hour per 25 sq. cms. area.
D_3	0.398	4.62 ± 0.05
G_3	0.517	4.62
	0.563	4.66
J_3	0.329	4.52

TABLE 2.

Leaves kept in dark for 24 hours. Intensity, 8,160 Lux. Temperature, 16·0° C. CO_2 content, 8·0% by volume.

Leaf.	Amt. of water (grms.).	Assimilation per hour per 25 sq. cms. area.
H_3	0.549	2.00 ± 0.05
	0.528	2.00
L_3	0.472	1.90
M_3	0.540	2.10
O_3	0.525	2.00

Each of the values for the assimilation is the mean of readings over six different periods. The probable error of each of these means is ±0·05.

These experiments show that the varying water content in different leaves had no influence on the rate of assimilation. In order to place the question of the relation between rate of assimilation and water content on a firmer basis experiments were carried out in two leaves, in each of which the water content was varied, and the rate of assimilation was determined at these varying water contents. The technique adopted was as follows:

Each leaf was kept in darkness for one day, for after this time the rate of assimilation has dropped to a value from which it falls only gradually on successive days. Over a period of three days (which was the usual time over which the experiments lasted) the drop in amount of assimilation is not great, and allowance can be made for it from other data obtained previously.

The rate of assimilation of the "normal" leaf (that is, the assimilation at the ordinary fresh weight of the leaf) was determined, and the leaf then placed under water in a vessel which could be evacuated. On admitting air into the evacuated vessel the intercellular spaces of the leaf become totally injected with

water. The leaf is then weighed, and its rate of assimilation determined. It is then allowed to dry out in the air, and the water content of the leaf observed at different intervals, and also determinations of the amount of photosynthesis made. Care is taken whilst measuring the amount of assimilation of the injected leaves that the amount of CO_2 in the apparatus remains constant, for it is here in relative minimum, and variations in its amounts causes variation in the amount of assimilation.

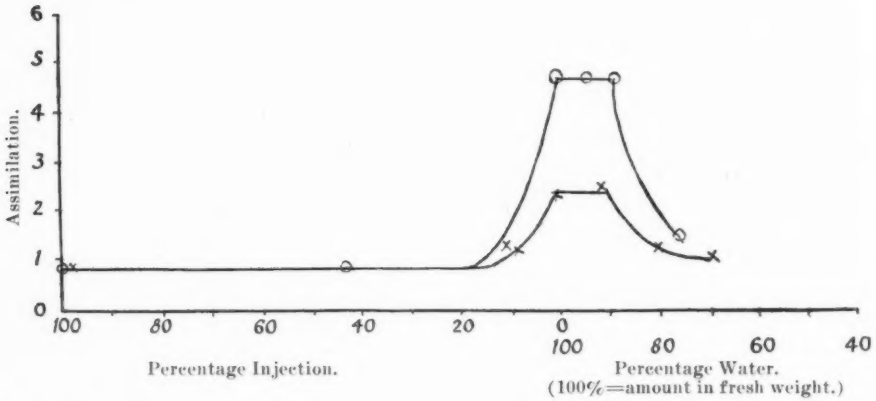

Fig. 1. Curve illustrating relation of amount of photosynthesis and water content of the leaf. The ordinate represents the rate of assimilation in ccs. per hour, and the abscissa the percentage injection of the leaf with water and the percentage of water in the leaf; the amount present in the fresh leaf being equal to 100%.

The leaf gradually returns to its "normal" value, and its rate of photosynthesis is again determined. Several determinations (e.g., leaf G3 and leaf H3) have shown that this rate, after de-injection, is the same as the originally determined rate. The leaf is then allowed to dry out from the normal value, and the amounts of water and of assimilation again determined at intervals. The results are shown in Table 3 and Table 4.

TABLE 3.

LEAF D_3.—Light, 27,500 Lux. CO_2, 10.5 ccs. Temperature, 16.0° C. Fresh weight, 0.641 grms. Dry weight, 0.243 grms. Totally injected weight, 1.233 grms.

Water content (grms.)	Per cent. injection.	Per cent. fresh weight.	Assimilation (ccs.).
0.592	100	—	0.80
0.478	43	—	0.80
0.392	—	100	4.62
0.362	—	91	4.60
0.348	—	89	4.50
0.300	—	76	1.41

TABLE 4.

LEAF D_3.—Light, 27,500 Lux. CO_2, 10.5 ccs. Temperature, 16.0° C. Fresh weight, 0.802 grms. Dry weight, 0.276 grms. Totally injected weight, 1.043 grms.

Water content (grms.)	Per cent. injection.	Per cent. fresh weight.	Assimilation (ccs.).
0.767	100	—	0.85
0.547	8.7	—	1.10
0.555	10.5	—	1.20
0.526	—	100	2.22
0.481	—	91.5	2.47
0.481	—	80.0	1.16
0.361	—	69.0	1.04

In this way one gets a complete picture of the relations between the water content and the rate of assimilation. As one would expect, whilst the leaf is injected the rate of assimilation is low, being limited by the slow diffusion of CO_2 in solution, and remains at this low value until nearly all the water of injection has disappeared from the intercellular spaces. The rate then rises rapidly to the "normal" value, and there remains constant over a period represented by a change in water content of about 10% of the fresh weight of the leaf.

The curves showing this change are plotted about the "normal" (fresh weight) value of the leaf. On the left-hand side of the normal are given the percentages injection (that is, increase in water above the "normal"), and on the right-hand side the actual percentages of water in the leaf, the amount of water in the fresh leaf being taken as 100%. On this side of the curve the rate of photosynthesis gradually drops, as has been observed previously.

REFERENCES.

1. Dastur, R. H.: Ann. of Bot., 39 (1925), p. 769.
2. Déhérain, P. P. and Maquenne, L.: Compt. rend., 103 (1886), p. 167.
3. Iljin, V. S.: Flora N.F., 16 (1923), p. 360.
4. Kreusler, V.: Landw. Jahrb., 14 (1885), p. 913.
5. Thoday, D.: Proc. Roy. Soc., B. 82 (1910), p. 421.

THE INFLUENCE OF MENSTRUATION ON GASEOUS METABOLISM

by

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(Submitted for publication 4th March, 1929.)

In the course of investigations cited in a previous paper (1), it was considered that, despite the existent conflict of opinion concerning the influence of menstruation upon the standard metabolism (2), an investigation was justified for the reason that there is clinical evidence of thyroid gland involvement with this function. This involvement is more especially noticeable in regions subject to endemic goitre. Further, the results of Wakeham seemed to point to a definite pre-menstrual rise (3), although his figures had not generally been accepted as indicating this, largely because the rise was apparent only by determining the average of a large number of individual determinations varying widely in their distribution about the mean. An attempt was made to extend Wakeham's results by a statistical analysis of a large number of estimations on women presenting themselves for clinical reasons, but it was found that no definite conclusion could be based upon such findings. It was decided to avoid the difficulties inherent in dealing with different subjects by making a close day-to-day study of two volunteers, and the results, which were obtained prior to receiving from Dr. Benedict an account of his own experience (4), justify, in view of the latter, their being placed on record.

Gas analysis methods had been used in the previous work, but in this the Benedict field apparatus was utilized, together with Dr. Benedict's method of plotting the results obtained (4). The subjects were examined before rising, and were in the post-absorptive state. It will be apparent from a survey of the figures, as well as by reference to the graphic presentation, that there is a definite pre-menstrual rise in the standard metabolism. In one instance the results are marred to a slight extent by the fact that the subject was at the time engaged in heavy social work, involving late hours and unaccustomed duties (bazaar work), and was, in our opinion, even clinically, not a good subject for such investigation at that period. See figure (1)*.

*NOTE.—The menstrual function in both these cases was unaccompanied by any physical discomfort or other disturbing factor.

Amounts of Oxygen Consumed in Estimation of E.C.M., æt. 34 yrs.

Aug.	17	228	c.c. per minute	Sep.	17	207	c.c. per minute
	18	226	" "		18	208	" "
	21	207	" "		19	241	" "
	22	201	" "		23	228	" "
	23	195	" "		24	242	" "
	25	208	" "		25	254	" "
	27	192	" "		26	248	" "
	28	184	" "		29	178	" "
	30	195	" "		30	193	" "
Sept.	1	216	" "	Oct.	2	197	" "
	3	221	" "		7	203	" "
	4	214	" "		8	190	" "
	6	222	" "		9	217	" "
	9	228	" "		10	228	" "
	11	236	" "		11	202	" "
	12	247	" "		12	195	" "
	14	217	" "		13	191	" "
	15	196	" "				

Amounts of Oxygen Consumed in Estimation of L.M., æt. 32 yrs.

(This subject went away on November 12th.)

Oct.	12	224	c.c. per minute	Oct.	26	201	c.c. per minute
	14	226	" "		28	205	" "
	15	208	" "		31	208	" "
	18	191	" "	Nov.	6	195	" "
	20	222	" "		8	216	" "
	21	203	" "		10	227	" "
	23	192	" "		11	230	" "
	25	196	" "		12	215	" "

SUMMARY.

From a study of two normal female subjects it is concluded that there is good evidence of a variation in metabolism during the menstrual cycle.

There is an immediately pre-menstrual rise in the standard metabolism, followed by a fall during the menstrual period.

The findings of Benedict are confirmed, and previous failures to detect the variation are believed to be due to the wide range of error introduced in the study of a large number of different subjects, as well as by the method of selecting single points in the menstrual cycle at which to make the estimation.

The thyroid enlargement noted, especially in goitre endemic regions, may be correlated with functional activity of the gland at the beginning of the menstrual period.

My thanks are due to Professor C. Stanton Hicks, who suggested this investigation, for his assistance throughout the work, and also to Dr. Francis Benedict for the loan of the apparatus used.

BIBLIOGRAPHY.

1. Matters, R. F.: "A Study of Calcium Metabolism in the Human Female," Aust. Journ. Exper. Biol. and Med. Sci.

2. DuBois, E. F.: "Basal Metabolism in Health and Disease," Lea and Febiger (Phil., 1924).
3. Wakeham, G.: "Basal Metabolism and the Menstrual Cycle," Journ. Biol. Chem., 56 (1923), p. 555.
4. Benedict, F. G.: "Normal Menstruation and Gaseous Metabolism," Amer. Journ. Physiol., 86 (1928), p. 59.
5. Benedict, F. G.: Boston Med. and Surg. Journ., 197 (1927).

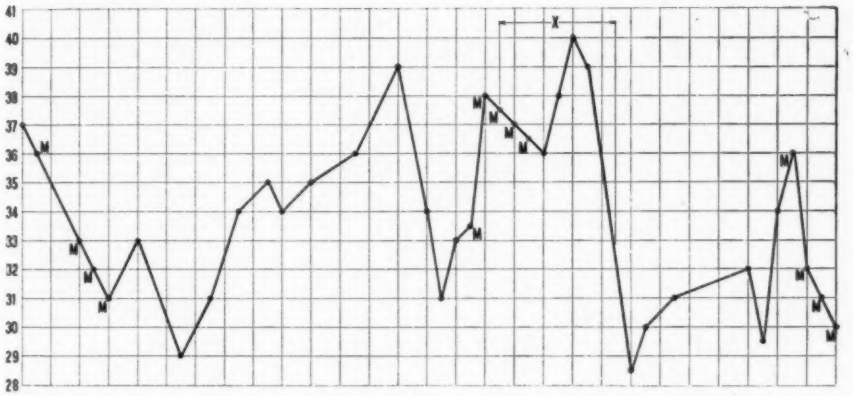

Fig. 1.

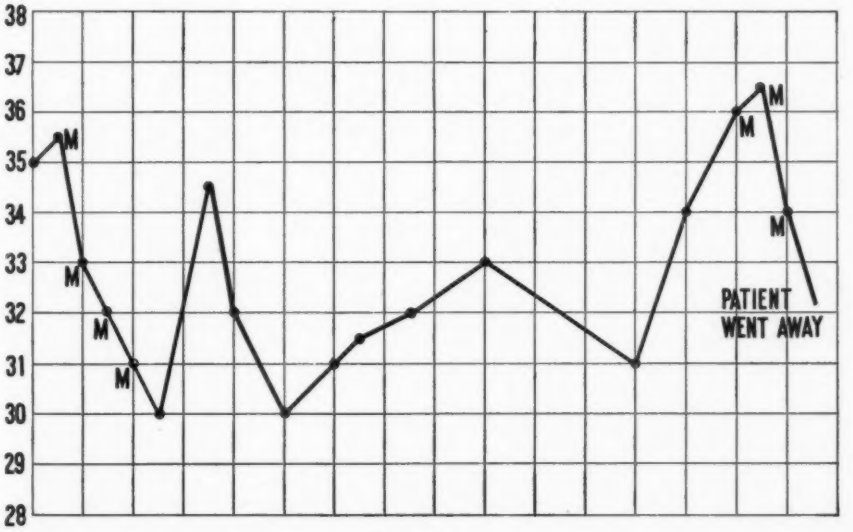

Fig. 2.